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(21) International Application Number: PCT/US93/01544 (22) International Filing Date: 18 February 1993 (18.02.93) (30) Priority data: 838,509 19 February 1992 (19.02.92) US (71) Applicant: THE STATE OF OREGON acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION ON BEHALF OF OREGON STATE UNIVERSITY [US/US]; Corvallis, OR 97331 (US). (72) Inventors: DOUGHERTY, William, G. ; 35163 Lillian Drive, Philomath, OR 97370 (US). LINDBO, John, A. ; 2910 N.W. Polk Street, #17, Corvallis, OR 97330 (US).		(74) Agents: POLLEY, Richard, J. et al.; Klarquist, Sparkman, Campbell, Leigh & Whinston, One World Trade Center, Suite 1600, 121 S.W. Salmon Street, Portland, OR 97204 (US). (81) Designated States: CA, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: PRODUCTION OF VIRAL RESISTANT PLANTS VIA INTRODUCTION OF UNTRANSLATABLE PLUS SENSE VIRAL RNA (57) Abstract Plants, such as tobacco, are made resistant to potyvirus infection by transformation with vectors which include a gene, derived from a potyvirus, mutated to encode an untranslatable plus sense RNA molecule. Mutagenized potyvirus genes and plant transformation vectors containing these genes are also disclosed.		

"Production of viral resistant plants via introduction of untranslatable plus sense viral RNA"

FIELD OF THE INVENTION

5 This invention is directed to the production of plants with a reduced susceptibility to virus infection.

BACKGROUND OF THE INVENTION

10 Plant viruses are responsible for major losses in worldwide crop production. Much effort is directed towards the development of new plant varieties which exhibit increased resistance to viral infection. Until recently such efforts were primarily based on the traditional plant breeding approach, however this approach is often limited by a lack of sources of resistance within the crop species. The advent of modern molecular biology techniques has facilitated the development of new methods of rendering plant varieties resistant to virus attack that are not limited by a requirement for preexisting resistance genes within a species.

Molecular Approaches

20 Many of these molecular approaches are based on the theory of pathogen derived resistance (Sanford and Johnston, 1985). This theory predicts that a "normal" host (plant) - pathogen (virus) relationship can be disrupted if the host organism expresses essential pathogen derived genes. It has been proposed that host organisms expressing pathogen gene products in excess amounts, at an inappropriate developmental stage, or in a dysfunctional form may disrupt the normal replicative cycle of the pathogen and result in an attenuated or aborted infection of the host.

30 Two approaches typify this pathogen derived resistance: coat protein mediated resistance and antisense RNA expression. It has been demonstrated that transgenic plants expressing a plant virus coat protein can be resistant to infection by the homologous virus. This coat protein mediated resistance has been

replication mechanism involves both translation of the plus sense RNA to yield viral gene products (which include a replicase and a proteinase) and also the synthesis of a minus sense RNA strand. This minus sense strand then acts as a template for the synthesis of many plus sense genomes which are subsequently encapsidated in coat protein to yield infectious mature "virions," thus completing the replicative cycle of the virus.

Experiments have been reported in which transgenic plants expressing the coat protein gene of a potyvirus show a reduced susceptibility to virus infection (Lawson et al. 1990; Ling et al. 1991; Stark and Beachy 1989).

SUMMARY OF THE INVENTION

The disclosed invention concerns a method of producing plants with a decreased susceptibility to virus infection. This is achieved by transforming plants with a DNA molecule which includes a gene derived in part from the genome of a plant virus. This gene is specifically constructed to produce an untranslatable version of a plus sense RNA molecule required for viral replication. Thus, expression of the gene within the plant causes the production of this non-functional molecule which then inhibits viral replication within the plant, rendering the plant resistant to viral infection.

In particular, invention provides an alternative and novel approach to rendering plants resistant to potyvirus infection.

Plants are transformed with a gene construct engineered to express an untranslatable form of the plus sense RNA which encodes the coat protein of a potyvirus.

In the case of Tobacco Etch Virus (TEV), it is demonstrated that tobacco plants transformed with such a gene construct accumulate the untranslatable plus sense RNA but do not produce detectable levels of the coat protein. It is further shown that these plants are resistant to TEV infection. It is also shown that

with the restriction enzyme *Nco*I, the DNA fragment delineated by the restriction enzyme sites at B and C was removed, and the plasmid religated to generate pTC:FL. pTC:FL contains the Tobacco Etch Virus (TEV) coat protein nucleotide sequence flanked by *Bam*HI restriction sites and the TEV 5' and 3' untranslated sequences (UTS). T7 and SP6 promoters are also shown. Abbreviations used in this diagram are as follows: T7, T7 RNA polymerase promoter sequence; SP6, SP6 RNA polymerase promoter sequence; ori, origin of replication; M13 ori, bacteriophage M13 single-stranded origin of replication; amp^r, β -lactamase gene. Lightly stippled areas are TEV 5' and 3' untranslated sequences; solid black area, TEV genome cDNA nucleotides 144 to 200; striped area, a portion of the TEV NIb gene (TEV nt 8462-8517); heavily stippled areas, cDNA of TEV CP nucleotide sequence (TEV nt 8518-9309).

Fig. 3 is a schematic representation of the forms of the Tobacco Etch Virus coat protein gene inserted into tobacco in the invention. All constructs contained the enhanced CaMV 35S (Enh 35S) promoter, CaMV 35S 5' untranslated sequence (UTS) of 50 bp and the CaMV 35S 3' UTS/polyadenylation site of 110 bp. The nomenclature used to describe the transgenic plant lines is presented along with the gene products produced in those plant lines (far right column). Abbreviations are as follows: 35S, transgenic plants containing the CaMV 35S promoter and 5' and 3' UTS only; FL, transgenic plants containing the transgene coding for full-length, AS and RC transgenic plants contain the transgene expressed as an antisense form of the TEV CP gene, or an untranslated sense form of the TEV CP gene, respectively. Stippled areas represent various forms of the TEV CP nucleotide sequence.

Fig. 4 is a graphic representation of the appearance of systemic symptoms in plants infected with Tobacco Etch Virus showing responses of control plants and transformed plants generated as described in the

Minus sense RNA: That form of RNA used as a template for plus sense RNA production.

Antisense RNA: RNA complementary to plus sense RNA form.

5 R₀ generation: Primary transformants.

 R₁ generation: Progeny of primary transformants.

 R₂ generation: Second generation progeny of R₀ generation (i.e., progeny of R₁ generation).

10 A gene derived in part from a plant virus RNA molecule: At least the portion of the gene encoding the untranslatable RNA molecule is derived from a plant virus RNA molecule.

GENERAL DESCRIPTION

15 An untranslatable plus sense RNA molecule is encoded by a gene located on the DNA molecule. The gene comprises DNA derived from a plant virus RNA genome and also DNA from heterologous sources. The DNA from heterologous sources includes elements controlling the
20 expression of the virus-derived DNA sequences. The DNA sequence of the gene is specifically altered so as to render the RNA molecule transcribed from the gene untranslatable. The presence of this untranslatable plus sense RNA within the cells of the transformed plant
25 reduces the susceptibility of the plant to viral infection.

 More particularly, the portion of the gene which comprises DNA from a plant virus has been derived from a potyvirus. Plants transformed with the DNA
30 molecule containing the gene are less susceptible to infection by potyviruses. Most specifically, the DNA from the potyvirus source has been derived from the coat protein gene of Tobacco Etch Virus and transformed plants are resistant to infection by Tobacco Etch Virus.
35 Plants which can be made resistant to potyvirus infection include, but are not limited to, tobacco.

 Accordingly, the present invention provides a method for genetically engineering plants by insertion,

experimental example given and will appreciate the wider potential utility of the invention.

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' nontranslated region which causes polyadenylate nucleotides to be added to the 3' end of the viral RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. Promoters which are known or are found to cause transcription of viral RNA in plant cells can be used in the present invention. Such promoters may be obtained from plants or viruses and include, but are not limited to, the CaMV 35S promoter. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of untranslatable plus sense RNA to render the plant substantially resistant to virus infection. The amount of untranslatable plus sense RNA needed to induce resistance may vary with the plant type. Accordingly, while the 35S promoter is preferred, it should be understood that this promoter may not be the optimal one for all embodiments of the present invention. Furthermore, the promoters used in the DNA constructs of the invention may be modified, if desired, to affect their control characteristics. DNA sequences have been identified which confer regulatory specificity on promoter regions. For example, the small subunit of the ribulose bis-phosphate carboxylase (ss

protein gene. Furthermore, it will be apparent that suitable genomic portions are not limited to complete gene sequences.

A disclosed embodiment of the invention
5 utilizes a double-stranded complementary DNA (cDNA)
derived from the region of the TEV genome encoding the
coat protein gene. To the 5' end of this cDNA is
ligated the CaMV 35S promoter and CaMV 35S RNA 5'
nontranslated region. To the 3' end is ligated the CaMV
10 35S 3' nontranslated region. These 5' and 3' sequences
are present to cause transcription of the gene in plant
cells by the cellular enzyme RNA polymerase to produce
an RNA molecule of sequence corresponding to the
sequence of the coat protein cDNA sequence. Ordinarily,
15 such an RNA would then be translated by ribosomes which
would synthesize a protein of amino acid sequence
specified by the nucleotide sequence of the RNA
molecule. Particular amino acids are specified by
nucleotide triplets termed codons. Codons which
20 stipulate translation initiation and termination are
also present in DNA and RNA sequences. The current
invention relates to RNA molecules which are
untranslatable by ribosomes. In the preferred
embodiment the sequence of the TEV cDNA encoding the
25 coat protein is mutated by a standard in vitro
mutagenesis technique to produce a frameshift mutation
early in the coat protein structural gene immediately
followed by three translation termination signal codons.
These mutations do not affect the ability of RNA
30 polymerase to transcribe an RNA molecule from the cDNA
but prevent translation of the transcribed RNA by
ribosomes. Those skilled in the art will recognize that
for the disclosed gene and for other genes, DNA
sequences can be altered in other ways to cause the DNA
35 to encode an untranslatable plus sense RNA molecule.
Thus the disclosed invention is not limited to the
mutations disclosed.

the TEV coat protein gene are combined in a single cloning vector. This vector is subsequently transformed into AT cells and the resultant cells are used to transform cultured tobacco cells.

5 Vectors suitable for the AT mediated transformation of plants with the DNA of the invention are disclosed. It will be obvious to one skilled in the art that a range of suitable vectors is available, including those disclosed by Bevan (1983),
10 Herrera-Estrella (1983), Klee (1985) and EPO publication 12,516 (Schilperoort et al.). Suitable vectors are available on a commercial basis from Clontech (Palo Alto, CA) and Pharmacia LKB (Pleasant Hill, CA) and other sources.

15 Following the transformation of plant cells and regeneration of transformed plants with the DNA molecules as described, regenerated plants are tested for increased virus resistance. Plants are preferably exposed to the virus at a concentration within a range
20 where the rate of disease development correlates linearly with virus concentration. Methods for virus inoculation are well known to those skilled in the art and are reviewed by Kado and Agrawai (1972). One such method includes abrading a leaf surface with an aqueous
25 suspension containing an abrasive material such as carborundrum and virus or dusting leaves with such an abrasive material and subsequently applying the virus onto the leaf surface. A virus suspension can be directly inoculated into leaf veins or alternatively
30 plants can be inoculated using insect vectors. The virus suspension may comprise purified virus particles, or alternatively, sap from virus infected plants may be utilized.

 Transformed plants are then assessed for
35 resistance to the virus. The assessment of resistance or reduced susceptibility may be manifest in different ways dependant on the particular virus type and plant type. Those skilled in the art will realize that a

cDNA. Plasmid DNA was isolated from colonies which hybridized with the probe, and the *SalI/EcoRI* cDNA inserts were sized by electrophoresis in a 0.8% (w/v) agarose gel using a horizontal water-cooled gel apparatus.

The *SalI/EcoRI* inserts from the recombinant molecules were isolated from an agarose gel with NA45 membrane (Schleicher & Schuell, Keene, NH) according to the manufacturer's protocol. The following restriction enzymes were used either alone or in combination to digest the isolated cDNA insert: *HindIII*, *XhoI*, *AluI*, *HaeIII*, *RsaI*, *Sau3A*, and *TaqI*. Restriction enzyme digestion products were inserted into the DNA of an appropriate M13 bacteriophage (Messing 1983) selected for the presence of corresponding polylinker restriction sites, and their nucleotide sequences were determined by dideoxy chain termination.

Plasmid pTL 37/8595 (Carrington and Dougherty 1987; Carrington et al. 1987, herein incorporated by reference) contains a cDNA copy of the genomic sequence of HAT TEV corresponding to nucleotides (nt) 1-200 and nt 8462-9495 (Fig. 2). (Numbering of the TEV genome nucleotides is according to that presented in Allison et al. 1986). The nucleotide sequence and deduced amino acid sequence of the Tobacco Etch Virus genome and the numbering system utilized by Allison et al. (1986) and herein is shown in Fig. 1 and SEQ ID No. 1 in the attached sequence listing. The first and last codons of the coat protein (CP) coding region in the TEV genome are nt 8518-8520 (encoding the amino acid serine) and 9307-9309 (opal stop codon) respectively. pTL 37/8595 was subject to *in vitro* site-directed mutagenesis as described by Taylor et al. (1985a, 1985b) herein incorporated by reference. In all cases, nucleotide changes were confirmed by dideoxy-nucleotide sequencing (Sanger et al. 1977).

TEV nt 9312-9317 were first mutated (Fig. 2) to generate a *BamHI* restriction site (GGATCC). TEV nt

no detectable protein products, while transcripts from pTC:FL produced proteins of the expected sizes.

The various forms of the CP nucleotide sequence were then inserted as *Bam*HI cassettes into the plant expression vector pPEV (see below and Fig. 3).

The full length TEV CP open reading frame of pTC:FL was inserted in the reverse orientation to make the antisense (AS) construct pTC:AS. The nucleotide sequence of the TEV CP gene in pTC:AS is shown in SEQ ID No. 4 in the attached sequence listing.

Transformation Vector Construction

Construction of pPEV. The vector pPEV is part of a binary vector system for *Agrobacterium tumefaciens* mediated plant cell transformation. Plasmid pPEV was constructed from the plasmids pCGN 2113 (Calgene), pCIB 710 and pCIB 200 (Ciba Geigy Corp.). pCGN 2113 contains the "enhanced" Cauliflower Mosaic Virus (CaMV) 35S promoter (CaMV sequences -941 to 90/-363 to +2, relative to the transcription start site) in a pUC derived plasmid backbone. pCIB 710 has been described (Rothstein et al. 1987) and pCIB 200 is a derivative of the wide host range plasmid pTJS 75 (Schmidhauser and Helinski 1985) which contains left and right *A. tumefaciens* T37 DNA borders, the plant selectable NOS/NPT II chimeric gene from the plasmid Bin 6 (Bevan 1984) and part of a pUC polylinker. The small *Eco*RI-*Eco*RV DNA fragment of pCIB 710 (Rothstein et al. 1987) was ligated into *Eco*RI-*Eco*RV digested pCGN 2113. This regenerated the enhanced CaMV 35S promoter (Kay et al. 1987) of pCGN 2113 and introduced the CaMV 35S 5' and 3' untranslated sequences into pCGN 2113. The CaMV 35S promoterterminator cassette of the resulting plasmid was isolated as an *Eco*RI-*Xba*I DNA fragment and ligated into *Eco*RI-*Xba*I digested pCIB 200 to generate pPEV. CP nucleotide sequences from PTC:FL, pTC:RC, and pTC:AS were cloned as *Bam*HI cassettes into *Bam*HI digested pPEV and orientation of inserts confirmed by digestion with appropriate restriction endonucleases.

isolated from tissue and RNA precipitated with LiCl as described by Verwoerd et al. (1989). RNAs were electrophoretically separated on 1.2% agarose gels containing 6% (v/v) formaldehyde and transferred to nitrocellulose. Prehybridization and hybridization conditions were as described in Sambrook et al. (1989). Strand specific riboprobes were generated from SP6 or T7 DNA dependent RNA polymerase transcription reactions of pTL 37/8595 linearized with the restriction enzymes Asp718 (Boehringer Mannheim, Indianapolis, IN) or HindIII, respectively, using α -labelled ^{32}P -CTP ribonucleotide and suggested procedures (Promega, Madison, WI).

An RNA transcript of approximately 1,000 nt was expected with all transgenic plant lines. Such a TEV CP transcript was detected in CP expressing plant lines by using a minus sense riboprobe containing the TEV CP sequence. A similar transcript was detected in AS plants by using a plus sense riboprobe containing the TEV CP sequence. The transcript in the RC line, while detected with a minus sense riboprobe, may have migrated as a slightly larger (ca 1,100-1,200 nt) RNA species, possibly due to termination at an alternately selected site and/or a longer poly-A tail on the transcript. Differing levels of CP transcript accumulation were observed among different transgenic plant lines. Transgenic plant lines expressing the coat protein of TEV were identified by western blot analysis using polyclonal antisera to TEV CP. Tissue samples of regenerated plants were ground in 10 volumes of 2X Laemmli (Tris-glycine) runner buffer (Laemmli 1970) and clarified by centrifugation in a microcentrifuge for 10 min. at 10,000xg. Protein concentration was estimated by the dye binding procedure of Bradford (1976) using BSA as a standard. Protein samples (50 μg total protein) were separated on a 12.5% polyacrylamide gel containing SDS and subjected to the immunoblot transfer procedures described by Towbin et al. (1979). Anti-TEV

rat (Fig. 4A). Plant lines which expressed FL TEV CP showed little or no delay in the appearance of symptoms when inoculated with infected plant sap. However, FL transgenic plants did show a slight attenuation of symptoms and eventually (2-4 weeks after initial appearance of symptoms), younger leaf tissue emerged devoid of symptoms and virus as demonstrated by back inoculation experiments. Typically chlorosis and etching on older systemic leaves was limited.

10 Ten independently transformed RC lines and seven independently transformed AS lines were obtained. Progeny from three of the RC lines, including line RC #5 and from one of the AS lines, including AS #3, showed an altered response to viral infection relative to control plants. All of these lines were verified to be transformed and were producing expected RNA products. A possible explanation for the variation in observed phenotype is the previously noted "position effect" whereby the expression of genes from identical DNA sequences integrated at different locations within the genome show varying patterns of tissue specificity.

20 Ten R2 expressing plants of the FL expressing line were inoculated with infected plant sap, and 20 R1 plants of lines AS #3 and RC #5 were inoculated with 50 μ l of a 5 μ g/ml solution of purified TEV. Identical results to those obtained by purified TEV inoculation were obtained when AS #3 and RC #5 R1 plants were inoculated with TEV-infected plant sap, as described above.

30 Transgenic Burley 49 plant lines AS #3 and RC #5, expressing only TEV CP related RNA sequences, showed a delay in the appearance of symptoms and a modification of symptoms when inoculated with TEV (Fig. 4B). Since the 20 R1 plants were not screened for expression of CP RNA prior to inoculation, some of the symptomatic plants represented non-expressing plants in which the gene of interest had been lost during Mendelian segregation. Modified symptoms on AS #3 plants appeared as small

and 5×10^4 extracted protoplasts were then subjected to western blot analysis as described above. Protoplast viability was measured by dye exclusion as described in Luciano et al. (1987). All electroporated protoplast samples had equivalent viability counts. The results indicated that protoplasts from all FL plant lines supported virus replication at levels comparable to wild type Burley 49 protoplasts. R1 transgenic plants from lines AS #3 and RC #5 were initially screened by northern analysis, and leaves from positive expressors were used in the production of protoplasts. Transfected protoplasts derived from AS #3 plants supported TEV replication, albeit at a reduced level. Protoplasts derived from RC #5 transgenic plant leaf tissue did not support TEV replication at a detectable level. These results, and those presented in the whole plant inoculation series, suggested AS and RC plants interfere with TEV replication.

Discussion of Data

The above example indicates that varying degrees of protection from TEV infection can be achieved by overexpression of coat protein and by expression of an antisense RNA. The current invention which comprises the expression of an untranslatable plus sense RNA molecule provides protection against TEV infection that is more effective than either of these two methods. Plants of line RC #5, transformed with the disclosed DNA molecule encoding an untranslatable plus sense RNA derived from the TEV coat protein gene, were asymptomatic and appear to be completely protected from virus infection. The disclosed invention therefore represents a new and effective way of generating potyvirus resistant germplasm.

Tobacco protoplasts derived from plants expressing the antisense RNA supported a reduced level of TEV replication compared to control cells derived from untransformed plants. In contrast, tobacco protoplasts derived from plants of line RC #5,

It will also be apparent to one skilled in the art that the described invention may also be used to produce plants resistant to viruses outside of the potyvirus family in instances where these viruses also produce a minus sense RNA replicative template.

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-27-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: William G. Dougherty and
John A. Lindbo
- 5 (ii) TITLE OF INVENTION: Production of Plants
Showing Immunity to Viral Infection via
Introduction of Genes Encoding Untranslatable
Plus Sense RNA Molecules
- (iii) NUMBER OF SEQUENCES: 4
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- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette, 5.25 inch
- 20 (B) COMPUTER: IBM PC Compatible
- (C) OPERATING SYSTEM: MS DOS
- (D) SOFTWARE: WordPerfect 5.1
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- (vii) ATTORNEY/AGENT INFORMATION
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- (2) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 9495
- (B) TYPE: Nucleic Acid
- 40 (C) STRANDEDNESS: Single

	AGC	GCA	CAT	ATG	GCT	GGA	GCG	AAT	GGA	AGC	ATT	TTG	AAG	AAG	GCA	GAA	270
	Ser	Ala	His	Met	Ala	Gly	Ala	Asn	Gly	Ser	Ile	Leu	Lys	Lys	Ala	Glu	
				30					35					40			
5	GAG	ACC	TCT	CGT	GCA	ATC	ATG	CAC	AAA	CCA	GTG	ATC	TTC	GGA	GAA	GAC	318
	Glu	Thr	Ser	Arg	Ala	Ile	Met	His	Lys	Pro	Val	Ile	Phe	Gly	Glu	Asp	
			45					50					55				
10	TAC	ATT	ACC	GAG	GCA	GAC	TTG	CCT	TAC	ACA	CCA	CTC	CAT	TTA	GAG	GTC	366
	Tyr	Ile	Thr	Glu	Ala	Asp	Leu	Pro	Tyr	Thr	Pro	Leu	His	Leu	Glu	Val	
		60					65					70					
15	GAT	GCT	GAA	ATG	GAG	CGG	ATG	TAT	TAT	CTT	GGT	CGT	CGC	GCG	CTC	ACC	414
	Asp	Ala	Glu	Met	Glu	Arg	Met	Tyr	Tyr	Leu	Gly	Arg	Arg	Ala	Leu	Thr	
	75					80					85					90	
20	CAT	GGC	AAG	AGA	CGC	AAA	GTT	TCT	GTG	AAT	AAC	AAG	AGG	AAC	AGG	AGA	462
	His	Gly	Lys	Arg	Arg	Lys	Val	Ser	Val	Asn	Asn	Lys	Arg	Asn	Arg	Arg	
					95					100					105		
25	AGG	AAA	GTG	GCC	AAA	ACG	TAC	GTG	GGG	CGT	GAT	TCC	ATT	GTT	GAG	AAG	510
	Arg	Lys	Val	Ala	Lys	Thr	Tyr	Val	Gly	Arg	Asp	Ser	Ile	Val	Glu	Lys	
				110					115					120			
30	ATT	GTA	GTG	CCC	CAC	ACC	GAG	AGA	AAG	GTT	GAT	ACC	ACA	GCA	GCA	GTG	558
	Ile	Val	Val	Pro	His	Thr	Glu	Arg	Lys	Val	Asp	Thr	Thr	Ala	Ala	Val	
				125				130					135				
35	GAA	GAC	ATT	TGC	AAT	GAA	GCT	ACC	ACT	CAA	CTT	GTG	CAT	AAT	AGT	ATG	606
	Glu	Asp	Ile	Cys	Asn	Glu	Ala	Thr	Thr	Gln	Leu	Val	His	Asn	Ser	Met	
		140					145					150					
40	CCA	AAG	CGT	AAG	AAG	CAG	AAA	AAC	TTC	TTG	CCC	GCC	ACT	TCA	CTA	AGT	654
	Pro	Lys	Arg	Lys	Lys	Gln	Lys	Asn	Phe	Leu	Pro	Ala	Thr	Ser	Leu	Ser	
	155					160					165					170	
45	AAC	GTG	TAT	GCC	CAA	ACT	TGG	AGC	ATA	GTG	CGC	AAA	CGC	CAT	ATG	CAG	702
	Asn	Val	Tyr	Ala	Gln	Thr	Trp	Ser	Ile	Val	Arg	Lys	Arg	His	Met	Gln	
					175					180					185		
50	GTG	GAG	ATC	ATT	AGC	AAG	AAG	AGC	GTC	CGA	GCG	AGG	GTC	AAG	AGA	TTT	750
	Val	Glu	Ile	Ile	Ser	Lys	Lys	Ser	Val	Arg	Ala	Arg	Val	Lys	Arg	Phe	
				190					195					200			
55	GAG	GGC	TCG	GTG	CAA	TTG	TTC	GCA	AGT	GTG	CGT	CAC	ATG	TAT	GGC	GAG	798
	Glu	Gly	Ser	Val	Gln	Leu	Phe	Ala	Ser	Val	Arg	His	Met	Tyr	Gly	Glu	
			205					210					215				
60	AGG	AAA	AGG	GTG	GAC	TTA	CGT	ATT	GAC	AAC	TGG	CAG	CAA	GAG	ACA	CTT	846
	Arg	Lys	Arg	Val	Asp	Leu	Arg	Ile	Asp	Asn	Trp	Gln	Gln	Glu	Thr	Leu	
			220				225					230					
65	CTA	GAC	CTT	GCT	AAA	AGA	TTT	AAG	AAT	GAG	AGA	GTG	GAT	CAA	TCG	AAG	894
	Leu	Asp	Leu	Ala	Lys	Arg	Phe	Lys	Asn	Glu	Arg	Val	Asp	Gln	Ser	Lys	
	235					240					245					250	
70	CTC	ACT	TTT	GGT	TCA	AGT	GGC	CTA	GTT	TTG	AGG	CAA	GGC	TCG	TAC	GGA	942
	Leu	Thr	Phe	Gly	Ser	Ser	Gly	Leu	Val	Leu	Arg	Gln	Gly	Ser	Tyr	Gly	
					255					260					265		
75	CCT	GCG	CAT	TGG	TAT	CGA	CAT	GGT	ATG	TTC	ATT	GTA	CGC	GGT	CGG	TCG	990
	Pro	Ala	His	Trp	Tyr	Arg	His	Gly	Met	Phe	Ile	Val	Arg	Gly	Arg	Ser	
				270					275					280			
80	GAT	GGG	ATG	TTG	GTG	GAT	GCT	CGT	GCG	AAG	GTA	ACG	TTC	GCT	GTT	TGT	1038
	Asp	Gly	Met	Leu	Val	Asp	Ala	Arg	Ala	Lys	Val	Thr	Phe	Ala	Val	Cys	
			285					290					295				

	ATT CAA GGC GAA ACT ATT GAG CGT AAA GAA ATT GGG AAT CAC TGC ATT	1902
	Ile Gln Gly Glu Thr Ile Glu Arg Lys Glu Ile Gly Asn His Cys Ile	
	575 580 585	
5	TCA ATG CGG AAT GGT AAT TAC GTG TAC CCA TGT TGT TGT GTT ACT CTT	1950
	Ser Met Arg Asn Gly Asn Tyr Val Tyr Pro Cys Cys Cys Val Thr Leu	
	590 595 600	
10	GAA GAT GGT AAG GCT CAA TAT TCG GAT CTA AAG CAC CCA ACG AAG AGA	1998
	Glu Asp Gly Lys Ala Gln Tyr Ser Asp Leu Lys His Pro Thr Lys Arg	
	605 610 615	
15	CAT CTG GTC ATT GGC AAC TCT GGC GAT TCA AAG TAC CTA GAC CTT CCA	2046
	His Leu Val Ile Gly Asn Ser Gly Asp Ser Lys Tyr Leu Asp Leu Pro	
	620 625 630	
20	GTT CTC AAT GAA GAG AAA ATG TAT ATA GCT AAT GAA GGT TAT TGC TAC	2094
	Val Leu Asn Glu Glu Lys Met Tyr Ile Ala Asn Glu Gly Tyr Cys Tyr	
	635 640 645 650	
25	ATG AAC ATT TTC TTT GCT CTA CTA GTG AAT GTC AAG GAA GAG GAT GCA	2142
	Met Asn Ile Phe Phe Ala Leu Leu Val Asn Val Lys Glu Glu Asp Ala	
	655 660 665	
30	AAG GAC TTC ACC AAG TTT ATA AGG GAC ACA ATT GTT CCA AAG CTT GGA	2190
	Lys Asp Phe Thr Lys Phe Ile Arg Asp Thr Ile Val Pro Lys Leu Gly	
	670 675 680	
35	GCG TGG CCA ACA ATG CAA GAT GTT GCA ACT GCA TGC TAC TTA CTT TCC	2238
	Ala Trp Pro Thr Met Gln Asp Val Ala Thr Ala Cys Tyr Leu Leu Ser	
	685 690 695	
40	ATT CTT TAC CCA GAT GTC CTG AGA GCT GAA CTA CCC AGA ATT TTG GTT	2286
	Ile Leu Tyr Pro Asp Val Leu Arg Ala Glu Leu Pro Arg Ile Leu Val	
	700 705 710	
45	GAT CAT GAC AAC AAA ACA ATG CAT GTT TTG GAT TCG TAT GGG TCT AGA	2334
	Asp His Asp Asn Lys Thr Met His Val Leu Asp Ser Tyr Gly Ser Arg	
	715 720 725 730	
50	ACG ACA GGA TAC CAC ATG TTG AAA ATG AAC ACA ACA TCC CAG CTA ATT	2382
	Thr Thr Gly Tyr His Met Leu Lys Met Asn Thr Thr Ser Gln Leu Ile	
	735 740 745	
55	GAA TTC GTT CAT TCA GGT TTG GAA TCC GAA ATG AAA ACT TAC AAT GTT	2430
	Glu Phe Val His Ser Gly Leu Glu Ser Glu Met Lys Thr Tyr Asn Val	
	750 755 760	
60	GGA GGG ATG AAC CGA GAT GTG GTC ACA CAA GGT GCA ATT GAG ATG TTG	2478
	Gly Gly Met Asn Arg Asp Val Val Thr Gln Gly Ala Ile Glu Met Leu	
	765 770 775	
65	ATC AAG TCT ATA TAC AAA CCA CAT CTC ATG AAG CAG TTA CTT GAG GAA	2526
	Ile Lys Ser Ile Tyr Lys Pro His Leu Met Lys Gln Leu Leu Glu Glu	
	780 785 790	
70	GAG CCA TAC ATA ATT GTC CTG GCA ATA GTC TCC CCT TCA ATT TTA ATT	2574
	Glu Pro Tyr Ile Ile Val Leu Ala Ile Val Ser Pro Ser Ile Leu Ile	
	795 800 805 810	
75	GCC ATG TAC AAC TCT GGA ACT TTT GAG CAG GCG TTA CAA ATG TGG TTG	2622
	Ala Met Tyr Asn Ser Gly Thr Phe Glu Gln Ala Leu Gln Met Trp Leu	
	815 820 825	
80	CCA AAT ACA ATG AGG TTA GCT AAC CTC GCT GCC ATC TTG TCA GCC TTA	2670
	Pro Asn Thr Met Arg Leu Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu	
	830 835 840	

	GAG ATA GCA TAC TTT GAA AAG ATT ATA GCT TTC ATC ACA CTT GTA TTA	3534
	Glu Ile Ala Tyr Phe Glu Lys Ile Ile Ala Phe Ile Thr Leu Val Leu	
	1115 1120 1125 1130	
5	ATG GCT TTT GAC GCT GAG CGG AGT GAT GGA GTG TTC AAG ATA CTC AAT	3582
	Met Ala Phe Asp Ala Glu Arg Ser Asp Gly Val Phe Lys Ile Leu Asn	
	1135 1140 1145	
10	AAG TTC AAA GGA ATA CTG AGC TCA ACG GAG AGG GAG ATC ATC TAC ACG	3630
	Lys Phe Lys Gly Ile Leu Ser Ser Thr Glu Arg Glu Ile Ile Tyr Thr	
	1150 1155 1160	
15	CAG AGT TTG GAT GAT TAC GTT ACA ACC TTT GAT GAC AAT ATG ACA ATC	3678
	Gln Ser Leu Asp Asp Tyr Val Thr Thr Phe Asp Asp Asn Met Thr Ile	
	1165 1170 1175	
20	AAC CTC GAG TTG AAT ATG GAT GAA CTC CAC AAG ACG AGC CTT CCT GGA	3726
	Asn Leu Glu Leu Asn Met Asp Glu Leu His Lys Thr Ser Leu Pro Gly	
	1180 1185 1190	
25	GTC ACT TTT AAG CAA TGG TGG AAC AAC CAA ATC AGC CGA GGC AAC GTG	3774
	Val Thr Phe Lys Gln Trp Trp Asn Asn Gln Ile Ser Arg Gly Asn Val	
	1195 1200 1205 1210	
30	AAG CCA CAT TAT AGA ACT GAG GGG CAC TTC ATG GAG TTT ACC AGA GAT	3822
	Lys Pro His Tyr Arg Thr Glu Gly His Phe Met Glu Phe Thr Arg Asp	
	1215 1220 1225	
35	ACT GCG GCA TCG GTT GCC AGC GAG ATA TCA CAC TCA CCC GCA AGA GAT	3870
	Thr Ala Ala Ser Val Ala Ser Glu Ile Ser His Ser Pro Ala Arg Asp	
	1230 1235 1240	
40	TTT CTT GTG AGA GGT GCT GTT GGA TCT GGA AAA TCC ACA GGA CTT CCA	3918
	Phe Leu Val Arg Gly Ala Val Gly Ser Gly Lys Ser Thr Gly Leu Pro	
	1245 1250 1255	
45	TAC CAT TTA TCA AAG AGA GGG AGA GTG TTA ATG CTT GAG CCT ACC AGA	3966
	Tyr His Leu Ser Lys Arg Gly Arg Val Leu Met Leu Glu Pro Thr Arg	
	1260 1265 1270	
50	CCA CTC ACA GAT AAC ATG CAC AAG CAA CTG AGA AGT GAA CCA TTT AAC	4014
	Pro Leu Thr Asp Asn Met His Lys Gln Leu Arg Ser Glu Pro Phe Asn	
	1275 1280 1285 1290	
55	TGC TTC CCA ACT TTG AGG ATG AGA GGG AAG TCA ACT TTT GGG TCA TCA	4062
	Cys Phe Pro Thr Leu Arg Met Arg Gly Lys Ser Thr Phe Gly Ser Ser	
	1295 1300 1305	
60	CCG ATC ACA GTC ATG ACT AGT GGA TTC GCT TTA CAC CAC TTT GCA CGA	4110
	Pro Ile Thr Val Met Thr Ser Gly Phe Ala Leu His His Phe Ala Arg	
	1310 1315 1320	
65	AAC ATA GCT GAG GTA AAA ACA TAC GAT TTT GTC ATA ATT GAT GAA TGT	4158
	Asn Ile Ala Glu Val Lys Thr Tyr Asp Phe Val Ile Ile Asp Glu Cys	
	1325 1330 1335	
70	CAT GTG AAT GAT GCT TCT GCT ATA GCG TTT AGG AAT CTA CTG TTT GAA	4206
	His Val Asn Asp Ala Ser Ala Ile Ala Phe Arg Asn Leu Leu Phe Glu	
	1340 1345 1350	
75	CAT GAA TTT GAA GGA AAA GTC CTC AAA GTG TCA GCC ACA CCA CCA GGT	4254
	His Glu Phe Glu Gly Lys Val Leu Lys Val Ser Ala Thr Pro Pro Gly	
	1355 1360 1365 1370	
80	AGA GAA GTT GAA TTT ACA ACT CAG TTT CCC GTG AAA CTC AAG ATA GAA	4302
	Arg Glu Val Glu Phe Thr Thr Gln Phe Pro Val Lys Leu Lys Ile Glu	
	1375 1380 1385	

	ATT CCA GAC TCC TTG CAT GAG GAA ATT TGG CAC ATT GTA GTC GCC CAT	5166
	Ile Pro Asp Ser Leu His Glu Glu Ile Trp His Ile Val Val Ala His	
	1660 1665 1670	
5	AAA GGT GAC TCG GGT ATT GGG AGG CTC ACT AGC GTA CAG GCA GCA AAG	5214
	Lys Gly Asp Ser Gly Ile Gly Arg Leu Thr Ser Val Gln Ala Ala Lys	
	1675 1680 1685 1690	
10	GTT GTT TAT ACT CTG CAA ACG GAT GTG CAC TCA ATT GCG AGG ACT CTA	5262
	Val Val Tyr Thr Leu Gln Thr Asp Val His Ser Ile Ala Arg Thr Leu	
	1695 1700 1705	
15	GCA TGC ATC AAT AGA CGC ATA GCA GAT GAA CAA ATG AAG CAG AGT CAT	5310
	Ala Cys Ile Asn Arg Arg Ile Ala Asp Glu Gln Met Lys Gln Ser His	
	1710 1715 1720	
20	TTT GAA GCC GCA ACT GGG AGA GCA TTT TCC TTC ACA AAT TAC TCA ATA	5358
	Phe Glu Ala Ala Thr Gly Arg Ala Phe Ser Phe Thr Asn Tyr Ser Ile	
	1725 1730 1735	
25	CAA AGC ATA TTT GAC ACG CTG AAA GCA AAT TAT GCT ACA AAG CAT ACG	5406
	Gln Ser Ile Phe Asp Thr Leu Lys Ala Asn Tyr Ala Thr Lys His Thr	
	1740 1745 1750	
30	AAA GAA AAT ATT GCA GTG CTT CAG CAG GCA AAA GAT CAA TTG CTA GAG	5454
	Lys Glu Asn Ile Ala Val Leu Gln Gln Ala Lys Asp Gln Leu Leu Glu	
	1755 1760 1765 1770	
35	TTT TCG AAC CTA GCA AAG GAT CAA GAT GTC ACG GGT ATC ATC CAA GAC	5502
	Phe Ser Asn Leu Ala Lys Asp Gln Asp Val Thr Gly Ile Ile Gln Asp	
	1775 1780 1785	
40	TTC AAT CAC CTG GAA ACT ATC TAT CTC CAA TCA GAT AGC GAA GTG GCT	5550
	Phe Asn His Leu Glu Thr Ile Tyr Leu Gln Ser Asp Ser Glu Val Ala	
	1790 1795 1800	
45	AAG CAT CTG AAG CTT AAA AGT CAC TGG AAT AAA AGC CAA ATC ACT AGG	5598
	Lys His Leu Lys Leu Lys Ser His Trp Asn Lys Ser Gln Ile Thr Arg	
	1805 1810 1815	
50	GAC ATC ATA ATA GCT TTG TCT GTG TTA ATT GGT GGT GGA TGG ATG CTT	5646
	Asp Ile Ile Ile Ala Leu Ser Val Leu Ile Gly Gly Gly Trp Met Leu	
	1820 1825 1830	
55	GCA ACG TAC TTC AAG GAC AAG TTC AAT GAA CCA GTC TAT TTC CAA GGG	5694
	Ala Thr Tyr Phe Lys Asp Lys Phe Asn Glu Pro Val Tyr Phe Gln Gly	
	1835 1840 1845 1850	
60	AAG AAG AAT CAG AAG CAC AAG CTT AAG ATG AGA GAG GCG CGT GGG GCT	5742
	Lys Lys Asn Gln Lys His Lys Leu Lys Met Arg Glu Ala Arg Gly Ala	
	1855 1860 1865	
65	AGA GGG CAA TAT GAG GTT GCA GCG GAG CCA GAG GCG CTA GAA CAT TAC	5790
	Arg Gly Gln Tyr Glu Val Ala Ala Glu Pro Glu Ala Leu Glu His Tyr	
	1870 1875 1880	
70	TTT GGA AGC GCA TAT AAT AAC AAA GGA AAG CGC AAG GGC ACC ACG AGA	5838
	Phe Gly Ser Ala Tyr Asn Asn Lys Gly Lys Arg Lys Gly Thr Thr Arg	
	1885 1890 1895	
75	GGA ATG GGT GCA AAG TCT CGG AAA TTC ATA AAC ATG TAT GGG TTT GAT	5886
	Gly Met Gly Ala Lys Ser Arg Lys Phe Ile Asn Met Tyr Gly Phe Asp	
	1900 1905 1910	
80	CCA ACT GAT TTT TCA TAC ATT AGG TTT GTG GAT CCA TTG ACA GGT CAC	5934
	Pro Thr Asp Phe Ser Tyr Ile Arg Phe Val Asp Pro Leu Thr Gly His	
	1915 1920 1925 1930	

	ATA CAC TCA GCA TCG AAT TTC ACC AAC ACA AAC AAT TAT TTC ACA AGC	6798
	Ile His Ser Ala Ser Asn Phe Thr Asn Thr Asn Asn Tyr Phe Thr Ser	
	2205 2210 2215	
5	GTG CCG AAA AAC TTC ATG GAA TTG TTG ACA AAT CAG GAG GCG CAG CAG	6846
	Val Pro Lys Asn Phe Met Glu Leu Leu Thr Asn Gln Glu Ala Gln Gln	
	2220 2225 2230	
10	TGG GTT AGT GGT TGG CGA TTA AAT GCT GAC TCA GTA TTG TGG GGG GGC	6894
	Trp Val Ser Gly Trp Arg Leu Asn Ala Asp Ser Val Leu Trp Gly Gly	
	2235 2240 2245 2250	
15	CAT AAA GTT TTC ATG AGC AAA CCT GAA GAG CCT TTT CAG CCA GTT AAG	6942
	His Lys Val Phe Met Ser Lys Pro Glu Glu Pro Phe Gln Pro Val Lys	
	2255 2260 2265	
	GAA GCG ACT CAA CTC ATG AAT GAA TTG GTG TAC TCG CAA GGG GAG AAG	6990
	Glu Ala Thr Gln Leu Met Asn Glu Leu Val Tyr Ser Gln Gly Glu Lys	
	2270 2275 2280	
20	AGG AAA TGG GTC GTG GAA GCA CTG TCA GGG AAC TTG AGG CCA GTG GCT	7038
	Arg Lys Trp Val Val Glu Ala Leu Ser Gly Asn Leu Arg Pro Val Ala	
	2285 2290 2295	
25	GAG TGT CCC AGT CAG TTA GTC ACA AAG CAT GTG GTT AAA GGA AAG TGT	7086
	Glu Cys Pro Ser Gln Leu Val Thr Lys His Val Val Lys Gly Lys Cys	
	2300 2305 2310	
30	CCC CTC TTT GAG CTC TAC TTG CAG TTG AAT CCA GAA AAG GAA GCA TAT	7134
	Pro Leu Phe Glu Leu Tyr Leu Gln Leu Asn Pro Glu Lys Glu Ala Tyr	
	2315 2320 2325 2330	
35	TTT AAA CCG ATG ATG GGA GCA TAT AAG CCA AGT CGA CTT AAT AGA GAG	7182
	Phe Lys Pro Met Met Gly Ala Tyr Lys Pro Ser Arg Leu Asn Arg Glu	
	2335 2340 2345	
	GCG TTC CTC AAG GAC ATT CTA AAA TAT GCT AGT GAA ATT GAG ATT GGG	7230
	Ala Phe Leu Lys Asp Ile Leu Lys Tyr Ala Ser Glu Ile Glu Ile Gly	
	2350 2355 2360	
40	AAT GTG GAT TGT GAC TTG CTG GAG CTT GCA ATA AGC ATG CTC GTC ACA	7278
	Asn Val Asp Cys Asp Leu Leu Glu Leu Ala Ile Ser Met Leu Val Thr	
	2365 2370 2375	
45	AAG CTC AAG GCG TTA GGA TTC CCA ACT GTG AAC TAC ATC ACT GAC CCA	7326
	Lys Leu Lys Ala Leu Gly Phe Pro Thr Val Asn Tyr Ile Thr Asp Pro	
	2380 2385 2390	
50	GAG GAA ATT TTT AGT GCA TTG AAT ATG AAA GCA GCT ATG GGA GCA CTA	7374
	Glu Glu Ile Phe Ser Ala Leu Asn Met Lys Ala Ala Met Gly Ala Leu	
	2395 2400 2405 2410	
55	TAC AAA GGC AAG AAG AAA GAA GCT CTC AGC GAG CTC ACA CTA GAT GAG	7422
	Tyr Lys Gly Lys Lys Lys Glu Ala Leu Ser Glu Leu Thr Leu Asp Glu	
	2415 2420 2425	
	CAG GAG GCA ATG CTC AAA GCA AGT TGC CTG CGA CTG TAT ACG GGA AAG	7470
	Gln Glu Ala Met Leu Lys Ala Ser Cys Leu Arg Leu Tyr Thr Gly Lys	
	2430 2435 2440	
60	TTG GGA ATT TGG AAT GGC TCA TTG AAA GCA GAG TTG CGT CCA ATT GAG	7518
	Leu Gly Ile Trp Asn Gly Ser Leu Lys Ala Glu Leu Arg Pro Ile Glu	
	2445 2450 2455	
65	AAG GTT GAA AAC AAC AAA ACG CGA ACT TTC ACA GCA GCA CCA ATA GAC	7566
	Lys Val Glu Asn Asn Lys Thr Arg Thr Phe Thr Ala Ala Pro Ile Asp	
	2460 2465 2470	

	AAG GCG CCA TAT CTG GCT GAG ACT GCG CTT AAG TTT TTG TAC ACA TCT	8430
	Lys Ala Pro Tyr Leu Ala Glu Thr Ala Leu Lys Phe Leu Tyr Thr Ser	
	2750 2755 2760	
5	CAG CAC GGA ACA AAC TCT GAG ATA GAA GAG TAT TTA AAA GTG TTG TAT	8478
	Gln His Gly Thr Asn Ser Glu Ile Glu Glu Tyr Leu Lys Val Leu Tyr	
	2765 2770 2775	
10	GAT TAC GAT ATT CCA ACG ACT GAG AAT CTT TAT TTT CAG AGT GGC ACT	8526
	Asp Tyr Asp Ile Pro Thr Thr Glu Asn Leu Tyr Phe Gln Ser Gly Thr	
	2780 2785 2790	
15	GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT	8574
	Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp	
	2795 2800 2805 2810	
	AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT	8622
	Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr	
	2815 2820 2825	
20	TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT	8670
	Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu	
	2830 2835 2840	
25	CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT	8718
	Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu	
	2845 2850 2855	
30	TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA	8766
	Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr	
	2860 2865 2870	
35	CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA	8814
	His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly	
	2875 2880 2885 2890	
	GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG	8862
	Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp	
	2895 2900 2905	
40	TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG	8910
	Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met	
	2910 2915 2920	
45	ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA	8958
	Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu	
	2925 2930 2935	
50	AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG	9006
	Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu	
	2940 2945 2950	
55	GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT	9054
	Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro	
	2955 2960 2965 2970	
	AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT	9102
	Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr	
	2975 2980 2985	
60	GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG	9150
	Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg	
	2990 2995 3000	
65	GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT	9198
	Glu Ala His Met Gln Met Lys Ala Ala Ala Val Arg Asn Ser Gly Thr	
	3005 3010 3015	

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Allison et al.
 (B) TITLE: The nucleotide sequence of the
 coding region of Tobacco Etch Virus
 Genomic RNA: Evidence for the
 Synthesis of a Single Polyprotein
 (C) JOURNAL: Virology
 (D) VOLUME: 154
 (E) ISSUE: --
 (F) PAGES: 9-20

(A) AUTHORS: Lindbo and Dougherty
 (B) TITLE: Untranslatable Transcripts of
 the tobacco etch virus coat protein
 gene sequence can interfere with
 tobacco etch virus replication in
 Transgenic Plants and Protoplasts
 (C) JOURNAL: Virology
 (D) VOLUME: 189
 (E) ISSUE: --
 (F) PAGES: 725-733

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

		ATG GGC ACT	9
		Met Gly Thr	
		1	
25			
	GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT	57	
	Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp		
	5 10 15		
30			
	AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT	105	
	Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr		
	20 25 30 35		
35			
	TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT	153	
	Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu		
	40 45 50		
40			
	CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT	201	
	Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu		
	55 60 65		
45			
	TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA	249	
	Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr		
	70 75 80		
50			
	CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA	297	
	His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly		
	85 90 95		
55			
	GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG	345	
	Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp		
	100 105 110 115		
55			
	TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG	393	
	Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met		
	120 125 130		

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(ix) FEATURE:

- 5 (A) NAME/KEY: Mutation of AGT-GGC
(Ser-Gly) to ATG-GCC (Met-Ser)
- (B) LOCATION: Nucleotides 1-6 of SEQ
ID NO. 3 (corresponding to nucleotides
8518-8523 of SEQ ID NO. 1)
- 10 (A) NAME/KEY: Frameshift mutation
(insertion of T) producing stop codon
- (B) LOCATION: Nucleotide 13 of SEQ ID
No. 3 (corresponding to position
between nucleotides 8529 and 8530 of
SEQ. ID No. 1)
- 15 (D) OTHER INFORMATION: SEQ ID No: 3 is
the modified Tobacco Etch Virus coat
protein gene present in pTC:RC.

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: J. A. Lindbo and
W. G. Dougherty
- 20 (B) TITLE: Pathogen-Derived Resistance to
a Potyvirus: Immune and Resistant
Phenotypes in Transgenic Tobacco
Expressing Altered Forms of a
Potyvirus Coat Protein Nucleotide
Sequence
- 25 (C) JOURNAL: Molecular Plant-Microbe
Interactions
- (D) VOLUME: 5
- (E) ISSUE: 2
- (F) PAGES: 144-153
- 30
- (A) AUTHORS: J. A. Lindbo and
W. G. Dougherty
- 35 (B) TITLE: Untranslatable Transcripts of
the Tobacco Etch Virus Coat Protein
Gene Sequence Can Interfere with
Tobacco Etch Virus Replication in
Transgenic Plants and Protoplasts
- (C) JOURNAL: Virology
- (D) VOLUME: 189
- 40 (E) ISSUE: --
- (F) PAGES: 725-733

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

	ATG GCC ACT	9
	Met Ser Thr	
45	GTG TGA TGA TGGTGCTAGC GCTGGTAAGA AGAAAGATCA AAAGGATGAT	58
	Val	

- (D) OTHER INFORMATION: SEQ ID No. 4 is the modified Tobacco Etch Virus Coat protein gene present in pTC:AS. It is the inverse complement of SEQ ID No. 2.

5 (x) PUBLICATION INFORMATION:

- (A) AUTHORS: J. A. Lindbo and W. G. Dougherty
- (B) TITLE: Untranslatable Transcripts of the Tobacco Etch Virus Coat Protein Gene Sequence Can Interfere with Tobacco Etch Virus Replication in Transgenic Plants and Protoplasts
- (C) JOURNAL: Virology
- (D) VOLUME: 189
- 15 (E) ISSUE: --
- (F) PAGES: 725-733

- (A) AUTHORS: J. A. Lindbo and W. G. Dougherty
- 20 (B) TITLE: Pathogen-Derived Resistance to a Potyvirus: Immune and Resistant Phenotypes in Transgenic Tobacco Expressing Altered Forms of a Potyvirus Coat Protein Nucleotide Sequence
- 25 (C) JOURNAL: Molecular Plant-Microbe Interactions
- (D) VOLUME: 5
- (E) ISSUE: 2
- (F) PAGES: 144-153

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

	TCACGTGGCGG ACCCCTAATA GTGTGTGCAT GTTACGGTTG ACATCGTGCG CTGTGTGGCG	60
	TTCAAGTGTCT TCCTCTGCAG TACCCACGTT GCCATCAAGA CCAAATAACC TAGTTCCACT	120
	GTTCGTACT GCAGCAGCTT TCATTTGCAT ATGCGCCTCC CTCGCTCTAA CAGGTGTTTT	180
	TGAAGTTAGC TCATAGAAGT CGAAGGCATA GCGTGACAAA CTCATGTCTG TAATGTTTTCT	240
35	CTGTAGACCA TACCTAGGCA TGTATGGTGG CTCCTATTTC CTCATCTCAA TATACGCTTC	300
	AGCCAGGTCA CTGAAGTGTG TCATAATTTG CCTCAGTGTG GGCTGCGCGT TTTCAACCAT	360
	TGGTTTCAGC GGGTATGAAA CTTGATCCTC ACCATCCATC ATAACCCAAG TTCCGTTCAA	420
	ATTTGGGGAA GTCCCATTTT CTATGCACCA CACCATAAAT CCATTAGCA ATATTTTCAT	480
	TTGCTCTTCA TCACTCCAT AGGGTGTGAT CACTGCCTGA TGCCACGCGG CAAACTGCTC	540
40	ATGTGTGGCT CGAGCATTTG ACAAATCAAT TTGCTGTGGG TTGTATCCTA AAAGGTGATT	600
	CAAGTTTACA ACCACCTCTC CCTCATCCT TGGATATTGA AGTTTTGTGG CCATAGCATT	660
	TATTCGTGGA ACTGAGAATG TTCTGGAAGT TCCAGCATTA ACATCCCTAT CCTTTGAAGC	720
	CTGCTCAGCG ACTTTATCAT CTTTTGATC TTTCTTCTTA CCAGCGTCAG CACCAGCATC	780
	CACAGTGCCC AT	792

15. A differentiated plant comprising transformed plant cells of claim 10.

16. A differentiated plant comprising transformed plant cells of claim 11.

5 17. A differentiated plant comprising transformed plant cells of claim 12.

18. A recombinant gene comprising:
control regions which regulate transcription of the
gene; and

10 a region, derived from a plant virus,
mutated so as to render the RNA transcribed from the
gene untranslatable.

19. The recombinant gene of claim 18 wherein
the plant virus is a potyvirus.

15 20. The recombinant gene of claim 19 wherein
the virus-derived region is derived from the region of
the viral genome encoding a coat protein.

21. The recombinant gene of claim 20 wherein
the potyvirus is Tobacco Etch Virus.

20 22. A method of producing plants with a
reduced susceptibility to viral infection, comprising:

forming a recombinant gene derived, in
part, from viral RNA wherein the gene is mutated to
encode an untranslatable plus sense RNA molecule; and
25 transforming plants with the recombinant
gene.

23. The method of claim 22 wherein the method
of producing plants comprises:

30 constructing a recombinant gene comprising
a region of a viral genome capable of being transcribed
in a plant;

mutating the recombinant gene to encode an
untranslatable plus sense RNA molecule;

35 cloning the recombinant untranslatable
gene into a plant transformation vector;

transforming plant cells with the
transformation vector; and

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NAAATAACAA ATCTCAACAC AACATATACA AAACAAACGA ATCTCAAGCA ATCAAGCATT	60
CTACTTCTAT TGCAGCAATT TAAATCATTT CTTTAAAGC AAAAGCAATT TTCTGAAAAT	120
TTTCACCATT TACGAACGAT AGCA ATG GCA CTG ATC TTT GGC ACA GTC AAC GCT	174
Met Ala Leu Ile Phe Gly Thr Val Asn Ala	10
1 5	
AAC ATC CTG AAG GAA GTG TTC GGT GGA GCT CGT ATG GCT TGC GTT ACC	222
Asn Ile Leu Lys Glu Val Phe Gly Gly Ala Arg Met Ala Cys Val Thr	25
15 20	
AGC GCA CAT ATG GCT GGA GCG AAT GGA AGC ATT TTG AAG AAG GCA GAA	270
Ser Ala His Met Ala Gly Ala Asn Gly Ser Ile Leu Lys Lys Ala Glu	40
30 35	
GAG ACC TCT CGT GCA ATC ATG CAC AAA CCA GTG ATC TTC GGA GAA GAC	318
Glu Thr Ser Arg Ala Ile Met His Lys Pro Val Ile Phe Gly Glu Asp	55
45 50	
TAC ATT ACC GAG GCA GAC TTG CCT TAC ACA CCA CTC CAT TTA GAG GTC	366
Tyr Ile Thr Glu Ala Asp Leu Pro Tyr Thr Pro Leu His Leu Glu Val	70
60 65	
GAT GCT GAA ATG GAG CGG ATG TAT TAT CTT GGT CGT CGC GCG CTC ACC	414
Asp Ala Glu Met Glu Arg Met Tyr Tyr Leu Gly Arg Arg Ala Leu Thr	90
75 80 85	
CAT GGC AAG AGA CGC AAA GTT TCT GTG AAT AAC AAG AGG AAC AGG AGA	462
His Gly Lys Arg Arg Lys Val Ser Val Asn Asn Lys Arg Asn Arg Arg	105
95 100	
AGG AAA GTG GCC AAA ACG TAC GTG GGG CGT GAT TCC ATT GTT GAG AAG	510
Arg Lys Val Ala Lys Thr Tyr Val Gly Arg Asp Ser Ile Val Glu Lys	120
110 115	
ATT GTA GTG CCC CAC ACC GAG AGA AAG GTT GAT ACC ACA GCA GCA GTG	558
Ile Val Val Pro His Thr Glu Arg Lys Val Asp Thr Thr Ala Ala Val	135
125 130	
GAA GAC ATT TGC AAT GAA GCT ACC ACT CAA CTT GTG CAT AAT AGT ATG	606
Glu Asp Ile Cys Asn Glu Ala Thr Thr Gln Leu Val His Asn Ser Met	150
140 145	
CCA AAG CGT AAG AAG CAG AAA AAC TTC TTG CCC GCC ACT TCA CTA AGT	654
Pro Lys Arg Lys Lys Gln Lys Asn Phe Leu Pro Ala Thr Ser Leu Ser	170
155 160 165	
AAC GTG TAT GCC CAA ACT TGG AGC ATA GTG CGC AAA CGC CAT ATG CAG	702
Asn Val Tyr Ala Gln Thr Trp Ser Ile Val Arg Lys Arg His Met Gln	185
175 180	
GTG GAG ATC ATT AGC AAG AAG AGC GTC CGA GCG AGG GTC AAG AGA TTT	750
Val Glu Ile Ile Ser Lys Lys Ser Val Arg Ala Arg Val Lys Arg Phe	200
190 195	
GAG GGC TCG GTG CAA TTG TTC GCA AGT GTG CGT CAC ATG TAT GGC GAG	798
Glu Gly Ser Val Gln Leu Phe Ala Ser Val Arg His Met Tyr Gly Glu	215
205 210	
AGG AAA AGG GTG GAC TTA CGT ATT GAC AAC TGG CAG CAA GAG ACA CTT	846
Arg Lys Arg Val Asp Leu Arg Ile Asp Asn Trp Gln Gln Glu Thr Leu	230
220 225	

FIG. 1

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CTA	GAC	CTT	GCT	AAA	AGA	TTT	AAG	AAT	GAG	AGA	GTG	GAT	CAA	TCG	AAG	894
Leu	Asp	Leu	Ala	Lys	Arg	Phe	Lys	Asn	Glu	Arg	Val	Asp	Gln	Ser	Lys	
235					240					245					250	
CTC	ACT	TTT	GGT	TCA	AGT	GGC	CTA	GTT	TTG	AGG	CAA	GGC	TCG	TAC	GGA	942
Leu	Thr	Phe	Gly	Ser	Ser	Gly	Leu	Val	Leu	Arg	Gln	Gly	Ser	Tyr	Gly	
				255					260					265		
CCT	GCG	CAT	TGG	TAT	CGA	CAT	GGT	ATG	TTC	ATT	GTA	CGC	GGT	CGG	TCG	990
Pro	Ala	His	Trp	Tyr	Arg	His	Gly	Met	Phe	Ile	Val	Arg	Gly	Arg	Ser	
			270					275					280			
GAT	GGG	ATG	TTG	GTG	GAT	GCT	CGT	GCG	AAG	GTA	ACG	TTC	GCT	GTT	TGT	1038
Asp	Gly	Met	Leu	Val	Asp	Ala	Arg	Ala	Lys	Val	Thr	Phe	Ala	Val	Cys	
		285					290					295				
CAC	TCA	ATG	ACA	CAT	TAT	AGC	GAC	AAA	TCA	ATC	TCT	GAG	GCA	TTC	TTC	1086
His	Ser	Met	Thr	His	Tyr	Ser	Asp	Lys	Ser	Ile	Ser	Glu	Ala	Phe	Phe	
	300					305					310					
ATA	CCA	TAC	TCT	AAG	AAA	TTC	TTG	GAG	TTG	AGA	CCA	GAT	GGA	ATC	TCC	1134
Ile	Pro	Tyr	Ser	Lys	Lys	Phe	Leu	Glu	Leu	Arg	Pro	Asp	Gly	Ile	Ser	
315					320					325					330	
CAT	GAG	TGT	ACA	AGA	GGA	GTA	TCA	GTT	GAG	CGG	TGC	GGT	GAG	GTG	GCT	1182
His	Glu	Cys	Thr	Arg	Gly	Val	Ser	Val	Glu	Arg	Cys	Gly	Glu	Val	Ala	
				335					340					345		
GCA	ATC	CTG	ACA	CAA	GCA	CTT	TCA	CCG	TGT	GGT	AAG	ATC	ACA	TGC	AAA	1230
Ala	Ile	Leu	Thr	Gln	Ala	Leu	Ser	Pro	Cys	Gly	Lys	Ile	Thr	Cys	Lys	
			350					355					360			
CGT	TGC	ATG	GTT	GAA	ACA	CCT	GAC	ATT	GTT	GAG	GGT	GAG	TCG	GGA	GAA	1278
Arg	Cys	Met	Val	Glu	Thr	Pro	Asp	Ile	Val	Glu	Gly	Glu	Ser	Gly	Glu	
		365					370					375				
AGT	GTC	ACC	AAC	CAA	GGT	AAG	CTC	CTA	GCA	ATG	CTG	AAA	GAA	CAG	TAT	1326
Ser	Val	Thr	Asn	Gln	Gly	Lys	Leu	Leu	Ala	Met	Leu	Lys	Glu	Gln	Tyr	
	380					385					390					
CCA	GAT	TTC	CCA	ATG	GCC	GAG	AAA	CTA	CTC	ACA	AGG	TTT	TTG	CAA	CAG	1374
Pro	Asp	Phe	Pro	Met	Ala	Glu	Lys	Leu	Leu	Thr	Arg	Phe	Leu	Gln	Gln	
395					400					405					410	
AAA	TCA	CTA	GTA	AAT	ACA	AAT	TTG	ACA	GCC	TGC	GTG	AGC	GTC	AAA	CAA	1422
Lys	Ser	Leu	Val	Asn	Thr	Asn	Leu	Thr	Ala	Cys	Val	Ser	Val	Lys	Gln	
				415					420					425		
CTC	ATT	GGT	GAC	CGC	AAA	CAA	GCT	CCA	TTC	ACA	CAC	GTA	CTG	GCT	GTC	1470
Leu	Ile	Gly	Asp	Arg	Lys	Gln	Ala	Pro	Phe	Thr	His	Val	Leu	Ala	Val	
			430					435					440			
AGC	GAA	ATT	CTG	TTT	AAA	GGC	AAT	AAA	CTA	ACA	GGG	GCT	GAT	CTC	GAA	1518
Ser	Glu	Ile	Leu	Phe	Lys	Gly	Asn	Lys	Leu	Thr	Gly	Ala	Asp	Leu	Glu	
		445					450					455				
GAG	GCA	AGC	ACA	CAT	ATG	CTT	GAA	ATA	GCA	AGG	TTC	TTG	AAC	AAT	CGC	1566
Glu	Ala	Ser	Thr	His	Met	Leu	Glu	Ile	Ala	Arg	Phe	Leu	Asn	Asn	Arg	
	460					465					470					
ACT	GAA	AAT	ATG	CGC	ATT	GGC	CAC	CTT	GGT	TCT	TTC	AGA	AAT	AAA	ATC	1614
Thr	Glu	Asn	Met	Arg	Ile	Gly	His	Leu	Gly	Ser	Phe	Arg	Asn	Lys	Ile	
475					480					485					490	

FIG. 1

TCA	TCG	AAG	GCC	CAT	GTG	AAT	AAC	GCA	CTC	ATG	TGT	GAT	AAT	CAA	CTT	1662
Ser	Ser	Lys	Ala	His	Val	Asn	Asn	Ala	Leu	Met	Cys	Asp	Asn	Gln	Leu	
				495					500					505		
GAT	CAG	AAT	GGG	AAT	TTT	ATT	TGG	GGA	CTA	AGG	GGT	GCA	CAC	GCA	AAG	1710
Asp	Gln	Asn	Gly	Asn	Phe	Ile	Trp	Gly	Leu	Arg	Gly	Ala	His	Ala	Lys	
			510					515					520			
AGG	TTT	CTT	AAA	GGA	TTT	TTC	ACT	GAG	ATT	GAC	CCA	AAT	GAA	GGA	TAC	1758
Arg	Phe	Leu	Lys	Gly	Phe	Phe	Thr	Glu	Ile	Asp	Pro	Asn	Glu	Gly	Tyr	
		525					530					535				
GAT	AAG	TAT	GTT	ATC	AGG	AAA	CAT	ATC	AGG	GGT	AGC	AGA	AAG	CTA	GCA	1806
Asp	Lys	Tyr	Val	Ile	Arg	Lys	His	Ile	Arg	Gly	Ser	Arg	Lys	Leu	Ala	
	540					545					550					
ATT	GGC	AAT	TTG	ATA	ATG	TCA	ACT	GAC	TTC	CAG	ACG	CTC	AGG	CAA	CAA	1854
Ile	Gly	Asn	Leu	Ile	Met	Ser	Thr	Asp	Phe	Gln	Thr	Leu	Arg	Gln	Gln	
555					560					565					570	
ATT	CAA	GGC	GAA	ACT	ATT	GAG	CGT	AAA	GAA	ATT	GGG	AAT	CAC	TGC	ATT	1902
Ile	Gln	Gly	Glu	Thr	Ile	Glu	Arg	Lys	Glu	Ile	Gly	Asn	His	Cys	Ile	
				575					580					585		
TCA	ATG	CGG	AAT	GGT	AAT	TAC	GTG	TAC	CCA	TGT	TGT	TGT	GTT	ACT	CTT	1950
Ser	Met	Arg	Asn	Gly	Asn	Tyr	Val	Tyr	Pro	Cys	Cys	Cys	Val	Thr	Leu	
			590					595					600			
GAA	GAT	GGT	AAG	GCT	CAA	TAT	TCG	GAT	CTA	AAG	CAC	CCA	ACG	AAG	AGA	1998
Glu	Asp	Gly	Lys	Ala	Gln	Tyr	Ser	Asp	Leu	Lys	His	Pro	Thr	Lys	Arg	
		605					610					615				
CAT	CTG	GTC	ATT	GGC	AAC	TCT	GGC	GAT	TCA	AAG	TAC	CTA	GAC	CTT	CCA	2046
His	Leu	Val	Ile	Gly	Asn	Ser	Gly	Asp	Ser	Lys	Tyr	Leu	Asp	Leu	Pro	
	620					625					630					
GTT	CTC	AAT	GAA	GAG	AAA	ATG	TAT	ATA	GCT	AAT	GAA	GGT	TAT	TGC	TAC	2094
Val	Leu	Asn	Glu	Glu	Lys	Met	Tyr	Ile	Ala	Asn	Glu	Gly	Tyr	Cys	Tyr	
635					640					645					650	
ATG	AAC	ATT	TTC	TTT	GCT	CTA	CTA	GTG	AAT	GTC	AAG	GAA	GAG	GAT	GCA	2142
Met	Asn	Ile	Phe	Phe	Ala	Leu	Leu	Val	Asn	Val	Lys	Glu	Glu	Asp	Ala	
				655					660					665		
AAG	GAC	TTC	ACC	AAG	TTT	ATA	AGG	GAC	ACA	ATT	GTT	CCA	AAG	CTT	GGA	2190
Lys	Asp	Phe	Thr	Lys	Phe	Ile	Arg	Asp	Thr	Ile	Val	Pro	Lys	Leu	Gly	
			670					675					680			
GCG	TGG	CCA	ACA	ATG	CAA	GAT	GTT	GCA	ACT	GCA	TGC	TAC	TTA	CTT	TCC	2238
Ala	Trp	Pro	Thr	Met	Gln	Asp	Val	Ala	Thr	Ala	Cys	Tyr	Leu	Leu	Ser	
		685					690					695				
ATT	CTT	TAC	CCA	GAT	GTC	CTG	AGA	GCT	GAA	CTA	CCC	AGA	ATT	TTG	GTT	2286
Ile	Leu	Tyr	Pro	Asp	Val	Leu	Arg	Ala	Glu	Leu	Pro	Arg	Ile	Leu	Val	
	700					705					710					
GAT	CAT	GAC	AAC	AAA	ACA	ATG	CAT	GTT	TTG	GAT	TCG	TAT	GGG	TCT	AGA	2334
Asp	His	Asp	Asn	Lys	Thr	Met	His	Val	Leu	Asp	Ser	Tyr	Gly	Ser	Arg	
	715				720					725					730	
ACG	ACA	GGA	TAC	CAC	ATG	TTG	AAA	ATG	AAC	ACA	ACA	TCC	CAG	CTA	ATT	2382
Thr	Thr	Gly	Tyr	His	Met	Leu	Lys	Met	Asn	Thr	Thr	Ser	Gln	Leu	Ile	
				735					740					745		

FIG. 1

GAA	TTC	GTT	CAT	TCA	GGT	TTG	GAA	TCC	GAA	ATG	AAA	ACT	TAC	AAT	GTT	2430
Glu	Phe	Val	His	Ser	Gly	Leu	Glu	Ser	Glu	Met	Lys	Thr	Tyr	Asn	Val	
			750					755					760			
GGA	GGG	ATG	AAC	CGA	GAT	GTG	GTC	ACA	CAA	GGT	GCA	ATT	GAG	ATG	TTG	2478
Gly	Gly	Met	Asn	Arg	Asp	Val	Val	Thr	Gln	Gly	Ala	Ile	Glu	Met	Leu	
		765					770					775				
ATC	AAG	TCT	ATA	TAC	AAA	CCA	CAT	CTC	ATG	AAG	CAG	TTA	CTT	GAG	GAA	2526
Ile	Lys	Ser	Ile	Tyr	Lys	Pro	His	Leu	Met	Lys	Gln	Leu	Leu	Glu	Glu	
	780					785					790					
GAG	CCA	TAC	ATA	ATT	GTC	CTG	GCA	ATA	GTC	TCC	CCT	TCA	ATT	TTA	ATT	2574
Glu	Pro	Tyr	Ile	Ile	Val	Leu	Ala	Ile	Val	Ser	Pro	Ser	Ile	Leu	Ile	
	795				800					805					810	
GCC	ATG	TAC	AAC	TCT	GGA	ACT	TTT	GAG	CAG	GCG	TTA	CAA	ATG	TGG	TTG	2622
Ala	Met	Tyr	Asn	Ser	Gly	Thr	Phe	Glu	Gln	Ala	Leu	Gln	Met	Trp	Leu	
			815						820					825		
CCA	AAT	ACA	ATG	AGG	TTA	GCT	AAC	CTC	GCT	GCC	ATC	TTG	TCA	GCC	TTA	2670
Pro	Asn	Thr	Met	Arg	Leu	Ala	Asn	Leu	Ala	Ala	Ile	Leu	Ser	Ala	Leu	
			830					835					840			
GCG	CAA	AAG	TTA	ACT	TTG	GCA	GAT	TTG	TTC	GTC	CAG	CAG	CGT	AAT	TTG	2718
Ala	Gln	Lys	Leu	Thr	Leu	Ala	Asp	Leu	Phe	Val	Gln	Gln	Arg	Asn	Leu	
		845					850					855				
ATT	AAT	GAG	TAT	GCG	CAG	GTA	ATT	TTG	GAC	AAT	CTG	ATT	GAC	GGT	GTC	2766
Ile	Asn	Glu	Tyr	Ala	Gln	Val	Ile	Leu	Asp	Asn	Leu	Ile	Asp	Gly	Val	
	860					865					870					
AGG	GTT	AAT	CAT	TCG	CTA	TCC	CTA	GCA	ATG	GAA	ATT	GTT	ACT	ATT	AAG	2814
Arg	Val	Asn	His	Ser	Leu	Ser	Leu	Ala	Met	Glu	Ile	Val	Thr	Ile	Lys	
	875				880					885					890	
CTG	GCC	ACC	CAA	GAG	ATG	GAC	ATG	GCG	TTG	AGG	GAA	GGT	GGC	TAT	GCT	2862
Leu	Ala	Thr	Gln	Glu	Met	Asp	Met	Ala	Leu	Arg	Glu	Gly	Gly	Tyr	Ala	
			895						900					905		
GTG	ACC	TCT	GAA	AAG	GTG	CAT	GAA	ATG	TTG	GAA	AAA	AAC	TAT	GTA	AAG	2910
Val	Thr	Ser	Glu	Lys	Val	His	Glu	Met	Leu	Glu	Lys	Asn	Tyr	Val	Lys	
			910					915					920			
GCT	TTG	AAG	GAT	GCA	TGG	GAC	GAA	TTA	ACT	TGG	TTG	GAA	AAA	TTC	TCC	2958
Ala	Leu	Lys	Asp	Ala	Trp	Asp	Glu	Leu	Thr	Trp	Leu	Glu	Lys	Phe	Ser	
		925					930					935				
GCA	ATC	AGG	CAT	TCA	AGA	AAG	CTC	TTG	AAA	TTT	GGG	CGA	AAG	CCT	TTA	3006
Ala	Ile	Arg	His	Ser	Arg	Lys	Leu	Leu	Lys	Phe	Gly	Arg	Lys	Pro	Leu	
	940					945					950					
ATC	ATG	AAA	AAC	ACC	GTA	GAT	TGC	GGC	GGA	CAT	ATA	GAC	TTG	TCT	GTG	3054
Ile	Met	Lys	Asn	Thr	Val	Asp	Cys	Gly	Gly	His	Ile	Asp	Leu	Ser	Val	
	955				960					965					970	
AAA	TCG	CTT	TTC	AAG	TTC	CAC	TTG	GAA	CTC	CTG	AAG	GGA	ACC	ATC	TCA	3102
Lys	Ser	Leu	Phe	Lys	Phe	His	Leu	Glu	Leu	Leu	Lys	Gly	Thr	Ile	Ser	
			975						980					985		
AGA	GCC	GTA	AAT	GGT	GGC	GCA	AGA	AAG	GTA	AGA	GTA	GCG	AAG	AAT	GCC	3150
Arg	Ala	Val	Asn	Gly	Gly	Ala	Arg	Lys	Val	Arg	Val	Ala	Lys	Asn	Ala	
			990					995					1000			

FIG. 1

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ATG	ACA	AAA	GGG	GTT	TTT	CTC	AAA	ATC	TAC	AGC	ATG	CTT	CCT	GAC	GTC	3198
Met	Thr	Lys	Gly	Val	Phe	Leu	Lys	Ile	Tyr	Ser	Met	Leu	Pro	Asp	Val	
		1005						1010				1015				
TAC	AAG	TTT	ATC	ACA	GTC	TCG	AGT	GTC	CTT	TCC	TTG	TTG	TTG	ACA	TTC	3246
Tyr	Lys	Phe	Ile	Thr	Val	Ser	Ser	Val	Leu	Ser	Leu	Leu	Leu	Thr	Phe	
	1020					1025					1030					
TTA	TTT	CAA	ATT	GAC	TGC	ATG	ATA	AGG	GCA	CAC	CGA	GAG	GCG	AAG	GTT	3294
Leu	Phe	Gln	Ile	Asp	Cys	Met	Ile	Arg	Ala	His	Arg	Glu	Ala	Lys	Val	
1035					1040					1045					1050	
GCT	GCA	CAG	TTG	CAG	AAA	GAG	AGC	GAG	TGG	GAC	AAT	ATC	ATC	AAT	AGA	3342
Ala	Ala	Gln	Leu	Gln	Lys	Glu	Ser	Glu	Trp	Asp	Asn	Ile	Ile	Asn	Arg	
			1055						1060					1065		
ACT	TTC	CAG	TAT	TCT	AAG	CTT	GAA	AAT	CCT	ATT	GGC	TAT	CGC	TCT	ACA	3390
Thr	Phe	Gln	Tyr	Ser	Lys	Leu	Glu	Asn	Pro	Ile	Gly	Tyr	Arg	Ser	Thr	
		1070						1075				1080				
GCG	GAG	GAA	AGA	CTC	CAA	TCA	GAA	CAC	CCC	GAG	GCT	TTC	GAG	TAC	TAC	3438
Ala	Glu	Glu	Arg	Leu	Gln	Ser	Glu	His	Pro	Glu	Ala	Phe	Glu	Tyr	Tyr	
	1085						1090					1095				
AAG	TTT	TGC	ATT	GGA	AAG	GAA	GAC	CTC	GTT	GAA	CAG	GCA	AAA	CAA	CCG	3486
Lys	Phe	Cys	Ile	Gly	Lys	Glu	Asp	Leu	Val	Glu	Gln	Ala	Lys	Gln	Pro	
	1100					1105					1110					
GAG	ATA	GCA	TAC	TTT	GAA	AAG	ATT	ATA	GCT	TTC	ATC	ACA	CTT	GTA	TTA	3534
Glu	Ile	Ala	Tyr	Phe	Glu	Lys	Ile	Ile	Ala	Phe	Ile	Thr	Leu	Val	Leu	
1115					1120					1125					1130	
ATG	GCT	TTT	GAC	GCT	GAG	CGG	AGT	GAT	GGA	GTG	TTC	AAG	ATA	CTC	AAT	3582
Met	Ala	Phe	Asp	Ala	Glu	Arg	Ser	Asp	Gly	Val	Phe	Lys	Ile	Leu	Asn	
			1135						1140					1145		
AAG	TTC	AAA	GGA	ATA	CTG	AGC	TCA	ACG	GAG	AGG	GAG	ATC	ATC	TAC	ACG	3630
Lys	Phe	Lys	Gly	Ile	Leu	Ser	Ser	Thr	Glu	Arg	Glu	Ile	Ile	Tyr	Thr	
		1150						1155					1160			
CAG	AGT	TTG	GAT	GAT	TAC	GTT	ACA	ACC	TTT	GAT	GAC	AAT	ATG	ACA	ATC	3678
Gln	Ser	Leu	Asp	Asp	Tyr	Val	Thr	Thr	Phe	Asp	Asp	Asn	Met	Thr	Ile	
	1165						1170					1175				
AAC	CTC	GAG	TTG	AAT	ATG	GAT	GAA	CTC	CAC	AAG	ACG	AGC	CTT	CCT	GGA	3726
Asn	Leu	Glu	Leu	Asn	Met	Asp	Glu	Leu	His	Lys	Thr	Ser	Leu	Pro	Gly	
	1180					1185					1190					
GTC	ACT	TTT	AAG	CAA	TGG	TGG	AAC	AAC	CAA	ATC	AGC	CGA	GGC	AAC	GTG	3774
Val	Thr	Phe	Lys	Gln	Trp	Trp	Asn	Asn	Gln	Ile	Ser	Arg	Gly	Asn	Val	
1195					1200					1205					1210	
AAG	CCA	CAT	TAT	AGA	ACT	GAG	GGG	CAC	TTC	ATG	GAG	TTT	ACC	AGA	GAT	3822
Lys	Pro	His	Tyr	Arg	Thr	Glu	Gly	His	Phe	Met	Glu	Phe	Thr	Arg	Asp	
			1215						1220					1225		
ACT	GCG	GCA	TCG	GTT	GCC	AGC	GAG	ATA	TCA	CAC	TCA	CCC	GCA	AGA	GAT	3870
Thr	Ala	Ala	Ser	Val	Ala	Ser	Glu	Ile	Ser	His	Ser	Pro	Ala	Arg	Asp	
			1230					1235					1240			
TTT	CTT	GTG	AGA	GGT	GCT	GTT	GGA	TCT	GGA	AAA	TCC	ACA	GGA	CTT	CCA	3918
Phe	Leu	Val	Arg	Gly	Ala	Val	Gly	Ser	Gly	Lys	Ser	Thr	Gly	Leu	Pro	
	1245						1250					1255				

FIG. 1

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TAC CAT TTA TCA AAG AGA GGG AGA GTG TTA ATG CTT GAG CCT ACC AGA	3966
Tyr His Leu Ser Lys Arg Gly Arg Val Leu Met Leu Glu Pro Thr Arg	
1260 1265 1270	
CCA CTC ACA GAT AAC ATG CAC AAG CAA CTG AGA AGT GAA CCA TTT AAC	4014
Pro Leu Thr Asp Asn Met His Lys Gln Leu Arg Ser Glu Pro Phe Asn	
1275 1280 1285 1290	
TGC TTC CCA ACT TTG AGG ATG AGA GGG AAG TCA ACT TTT GGG TCA TCA	4062
Cys Phe Pro Thr Leu Arg Met Arg Gly Lys Ser Thr Phe Gly Ser Ser	
1295 1300 1305	
CCG ATC ACA GTC ATG ACT AGT GGA TTC GCT TTA CAC CAC TTT GCA CGA	4110
Pro Ile Thr Val Met Thr Ser Gly Phe Ala Leu His His Phe Ala Arg	
1310 1315 1320	
AAC ATA GCT GAG GTA AAA ACA TAC GAT TTT GTC ATA ATT GAT GAA TGT	4158
Asn Ile Ala Glu Val Lys Thr Tyr Asp Phe Val Ile Ile Asp Glu Cys	
1325 1330 1335	
CAT GTG AAT GAT GCT TCT GCT ATA GCG TTT AGG AAT CTA CTG TTT GAA	4206
His Val Asn Asp Ala Ser Ala Ile Ala Phe Arg Asn Leu Leu Phe Glu	
1340 1345 1350	
CAT GAA TTT GAA GGA AAA GTC CTC AAA GTG TCA GCC ACA CCA CCA GGT	4254
His Glu Phe Glu Gly Lys Val Leu Lys Val Ser Ala Thr Pro Pro Gly	
1355 1360 1365 1370	
AGA GAA GTT GAA TTT ACA ACT CAG TTT CCC GTG AAA CTC AAG ATA GAA	4302
Arg Glu Val Glu Phe Thr Thr Gln Phe Pro Val Lys Leu Lys Ile Glu	
1375 1380 1385	
GAG GCT CTT AGC TTT CAG GAA TTT GTA AGT TTA CAA GGG ACA GGT GCC	4350
Glu Ala Leu Ser Phe Gln Glu Phe Val Ser Leu Gln Gly Thr Gly Ala	
1390 1395 1400	
AAC GCC GAT GTG ATT AGT TGT GGC GAC AAC ATA CTA GTA TAT GTT GCT	4398
Asn Ala Asp Val Ile Ser Cys Gly Asp Asn Ile Leu Val Tyr Val Ala	
1405 1410 1415	
AGC TAC AAT GAT GTT GAT AGT CTT GGC AAG CTC CTT GTG CAA AAG GGA	4446
Ser Tyr Asn Asp Val Asp Ser Leu Gly Lys Leu Leu Val Gln Lys Gly	
1420 1425 1430	
TAC AAA GTG TCG AAG ATT GAT GGA AGA ACA ATG AAG AGT GGA GGA ACT	4494
Tyr Lys Val Ser Lys Ile Asp Gly Arg Thr Met Lys Ser Gly Gly Thr	
1435 1440 1445 1450	
GAA ATA ATC ACT GAA GGT ACT TCA GTG AAA AAG CAT TTC ATA GTC GCA	4542
Glu Ile Ile Thr Glu Gly Thr Ser Val Lys Lys His Phe Ile Val Ala	
1455 1460 1465	
ACT AAC ATT ATT GAG AAT GGT GTA ACC ATT GAC ATT GAT GTA GTT GTG	4590
Thr Asn Ile Ile Glu Asn Gly Val Thr Ile Asp Ile Asp Val Val Val	
1470 1475 1480	
GAT TTT GGG ACT AAG GTT GTA CCA GTT TTG GAT GTG GAC AAT AGA GCG	4638
Asp Phe Gly Thr Lys Val Val Pro Val Leu Asp Val Asp Asn Arg Ala	
1481 1490 1495	
GTG CAG TAC AAC AAA ACT GTG GTG AGT TAT GGG GAG CGC ATC CAA AAA	4686
Val Gln Tyr Asn Lys Thr Val Val Ser Tyr Gly Glu Arg Ile Gln Lys	
1500 1505 1510	

FIG. 1

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CTC	GGT	AGA	GTT	GGG	CGA	CAC	AAG	GAA	GGA	GTA	GCA	CTT	CGA	ATT	GGC	4734
Leu	Gly	Arg	Val	Gly	Arg	His	Lys	Glu	Gly	Val	Ala	Leu	Arg	Ile	Gly	
1515					1520					1525					1530	
CAA	ACA	AAT	AAA	ACA	CTG	GTT	GAA	ATT	CCA	GAA	ATG	GTT	GCC	ACT	GAA	4782
Gln	Thr	Asn	Lys	Thr	Leu	Val	Glu	Ile	Pro	Glu	Met	Val	Ala	Thr	Glu	
				1535					1540						1545	
GCT	GCC	TTT	CTA	TGC	TTC	ATG	TAC	AAT	TTG	CCA	GTG	ACA	ACA	CAG	AGT	4830
Ala	Ala	Phe	Leu	Cys	Phe	Met	Tyr	Asn	Leu	Pro	Val	Thr	Thr	Gln	Ser	
			1550					1555							1560	
GTT	TCA	ACC	ACA	CTG	CTG	GAA	AAT	GCC	ACA	TTA	TTA	CAA	GCT	AGA	ACT	4878
Val	Ser	Thr	Thr	Leu	Leu	Glu	Asn	Ala	Thr	Leu	Leu	Gln	Ala	Arg	Thr	
		1565					1570						1575			
ATG	GCA	CAG	TTT	GAG	CTA	TCA	TAT	TTT	TAC	ACA	ATT	AAT	TTT	GTG	CGA	4926
Met	Ala	Gln	Phe	Glu	Leu	Ser	Tyr	Phe	Tyr	Thr	Ile	Asn	Phe	Val	Arg	
	1580					1585					1590					
TTT	GAT	GGT	AGT	ATG	CAT	CCA	GTC	ATA	CAT	GAC	AAG	CTG	AAG	CGC	TTT	4974
Phe	Asp	Gly	Ser	Met	His	Pro	Val	Ile	His	Asp	Lys	Leu	Lys	Arg	Phe	
1595					1600					1605					1610	
AAG	CTA	CAC	ACT	TGT	GAG	ACA	TTC	CTC	AAT	AAG	TTG	GCG	ATC	CCA	AAT	5022
Lys	Leu	His	Thr	Cys	Glu	Thr	Phe	Leu	Asn	Lys	Leu	Ala	Ile	Pro	Asn	
				1615					1620						1625	
AAA	GGC	TTA	TCC	TCT	TGG	CTT	ACG	AGT	GGA	GAG	TAT	AAG	CGA	CTT	GGT	5070
Lys	Gly	Leu	Ser	Ser	Trp	Leu	Thr	Ser	Gly	Glu	Tyr	Lys	Arg	Leu	Gly	
			1630					1635					1640			
TAC	ATA	GCA	GAG	GAT	GCT	GGC	ATA	AGA	ATC	CCA	TTC	GTG	TGC	AAA	GAA	5118
Tyr	Ile	Ala	Glu	Asp	Ala	Gly	Ile	Arg	Ile	Pro	Phe	Val	Cys	Lys	Glu	
		1645					1650						1655			
ATT	CCA	GAC	TCC	TTG	CAT	GAG	GAA	ATT	TGG	CAC	ATT	GTA	GTC	GCC	CAT	5166
Ile	Pro	Asp	Ser	Leu	His	Glu	Glu	Ile	Trp	His	Ile	Val	Val	Ala	His	
	1660					1665					1670					
AAA	GGT	GAC	TCG	GGT	ATT	GGG	AGG	CTC	ACT	AGC	GTA	CAG	GCA	GCA	AAG	5214
Lys	Gly	Asp	Ser	Gly	Ile	Gly	Arg	Leu	Thr	Ser	Val	Gln	Ala	Ala	Lys	
1675					1680					1685					1690	
GTT	GTT	TAT	ACT	CTG	CAA	ACG	GAT	GTG	CAC	TCA	ATT	GCG	AGG	ACT	CTA	5262
Val	Val	Tyr	Thr	Leu	Gln	Thr	Asp	Val	His	Ser	Ile	Ala	Arg	Thr	Leu	
				1695					1700						1705	
GCA	TGC	ATC	AAT	AGA	CGC	ATA	GCA	GAT	GAA	CAA	ATG	AAG	CAG	AGT	CAT	5310
Ala	Cys	Ile	Asn	Arg	Arg	Ile	Ala	Asp	Glu	Gln	Met	Lys	Gln	Ser	His	
			1710					1715							1720	
TTT	GAA	GCC	GCA	ACT	GGG	AGA	GCA	TTT	TCC	TTC	ACA	AAT	TAC	TCA	ATA	5358
Phe	Glu	Ala	Ala	Thr	Gly	Arg	Ala	Phe	Ser	Phe	Thr	Asn	Tyr	Ser	Ile	
		1725					1730						1735			
CAA	AGC	ATA	TTT	GAC	ACG	CTG	AAA	GCA	AAT	TAT	GCT	ACA	AAG	CAT	ACG	5406
Gln	Ser	Ile	Phe	Asp	Thr	Leu	Lys	Ala	Asn	Tyr	Ala	Thr	Lys	His	Thr	
		1740				1745					1750					
AAA	GAA	AAT	ATT	GCA	GTG	CTT	CAG	CAG	GCA	AAA	GAT	CAA	TTG	CTA	GAG	5454
Lys	Glu	Asn	Ile	Ala	Val	Leu	Gln	Gln	Ala	Lys	Asp	Gln	Leu	Leu	Glu	
1755					1760					1765					1770	

FIG. 1

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TTT TCG AAC CTA GCA AAG GAT CAA GAT GTC ACG GGT ATC ATC CAA GAC	5502
Phe Ser Asn Leu Ala Lys Asp Gln Asp Val Thr Gly Ile Ile Gln Asp	
1775 1780 1785	
TTC AAT CAC CTG GAA ACT ATC TAT CTC CAA TCA GAT AGC GAA GTG GCT	5550
Phe Asn His Leu Glu Thr Ile Tyr Leu Gln Ser Asp Ser Glu Val Ala	
1790 1795 1800	
AAG CAT CTG AAG CTT AAA AGT CAC TGG AAT AAA AGC CAA ATC ACT AGG	5598
Lys His Leu Lys Leu Lys Ser His Trp Asn Lys Ser Gln Ile Thr Arg	
1805 1810 1815	
GAC ATC ATA ATA GCT TTG TCT GTG TTA ATT GGT GGT GGA TGG ATG CTT	5646
Asp Ile Ile Ile Ala Leu Ser Val Leu Ile Gly Gly Gly Trp Met Leu	
1820 1825 1830	
GCA ACG TAC TTC AAG GAC AAG TTC AAT GAA CCA GTC TAT TTC CAA GGG	5694
Ala Thr Tyr Phe Lys Asp Lys Phe Asn Glu Pro Val Tyr Phe Gln Gly	
1835 1840 1845 1850	
AAG AAG AAT CAG AAG CAC AAG CTT AAG ATG AGA GAG GCG CGT GGG GCT	5742
Lys Lys Asn Gln Lys His Lys Leu Lys Met Arg Glu Ala Arg Gly Ala	
1855 1860 1865	
AGA GGG CAA TAT GAG GTT GCA GCG GAG CCA GAG GCG CTA GAA CAT TAC	5790
Arg Gly Gln Tyr Glu Val Ala Ala Glu Pro Glu Ala Leu Glu His Tyr	
1870 1875 1880	
TTT GGA AGC GCA TAT AAT AAC AAA GGA AAG CGC AAG GGC ACC ACG AGA	5838
Phe Gly Ser Ala Tyr Asn Asn Lys Gly Lys Arg Lys Gly Thr Thr Arg	
1885 1890 1895	
GGA ATG GGT GCA AAG TCT CGG AAA TTC ATA AAC ATG TAT GGG TTT GAT	5886
Gly Met Gly Ala Lys Ser Arg Lys Phe Ile Asn Met Tyr Gly Phe Asp	
1900 1905 1910	
CCA ACT GAT TTT TCA TAC ATT AGG TTT GTG GAT CCA TTG ACA GGT CAC	5934
Pro Thr Asp Phe Ser Tyr Ile Arg Phe Val Asp Pro Leu Thr Gly His	
1915 1920 1925 1930	
ACT ATT GAT GAG TCC ACA AAC GCA CCT ATT GAT TTA GTG CAG CAT GAG	5982
Thr Ile Asp Glu Ser Thr Asn Ala Pro Ile Asp Leu Val Gln His Glu	
1935 1940 1945	
TTT GGA AAG GTT AGA ACA CGC ATG TTA ATT GAC GAT GAG ATA GAG CCT	6030
Phe Gly Lys Val Arg Thr Arg Met Leu Ile Asp Asp Glu Ile Glu Pro	
1950 1955 1960	
CAA AGT CTT AGC ACC CAC ACC ACA ATC CAT GCT TAT TTG GTG AAT AGT	6078
Gln Ser Leu Ser Thr His Thr Thr Ile His Ala Tyr Leu Val Asn Ser	
1965 1970 1975	
GGC ACG AAG AAA GTT CTT AAG GTT GAT TTA ACA CCA CAC TCG TCG CTA	6126
Gly Thr Lys Lys Val Leu Lys Val Asp Leu Thr Pro His Ser Ser Leu	
1980 1985 1990	
CGT GCG AGT GAG AAA TCA ACA GCA ATA ATG GGA TTT CCT GAA AGG GAG	6174
Arg Ala Ser Glu Lys Ser Thr Ala Ile Met Gly Phe Pro Glu Arg Glu	
1995 2000 2005 2010	
AAT GAA TTG CGT CAA ACC GGC ATG GCA GTG CCA GTG GCT TAT GAT CAA	6222
Asn Glu Leu Arg Gln Thr Gly Met Ala Val Pro Val Ala Tyr Asp Gln	
2015 2020 2025	

FIG. 1

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TTG CCA CCA AAG AAT GAG GAC TTG ACG TTT GAA GGA GAA AGC TTG TTT Leu Pro Pro Lys Asn Glu Asp Leu Thr Phe Glu Gly Glu Ser Leu Phe 2030 2035 2040	6270
AAG GGA CCA CGT GAT TAC AAC CCG ATA TCG AGC ACC ATT TGT CAT TTG Lys Gly Pro Arg Asp Tyr Asn Pro Ile Ser Ser Thr Ile Cys His Leu 2045 2050 2055	6318
ACG AAT GAA TCT GAT GGG CAC ACA ACA TCG TTG TAT GGT ATT GGA TTT Thr Asn Glu Ser Asp Gly His Thr Thr Ser Leu Tyr Gly Ile Gly Phe 2060 2065 2070	6366
GGT CCC TTC ATC ATT ACA AAC AAG CAC TTG TTT AGA AGA AAT AAT GGA Gly Pro Phe Ile Ile Thr Asn Lys His Leu Phe Arg Arg Asn Asn Gly 2075 2080 2085 2090	6414
ACA CTG TTG GTC CAA TCA CTA CAT GGT GTA TTC AAG GTC AAG AAC ACC Thr Leu Leu Val Gln Ser Leu His Gly Val Phe Lys Val Lys Asn Thr 2095 2100 2105	6462
ACG ACT TTG CAA CAA CAC CTC ATT GAT GGG AGG GAC ATG ATA ATT ATT Thr Thr Leu Gln Gln His Leu Ile Asp Gly Arg Asp Met Ile Ile Ile 2110 2115 2120	6510
CGC ATG CCT AAG GAT TTC CCA CCA TTT CCT CAA AAG CTG AAA TTT AGA Arg Met Pro Lys Asp Phe Pro Pro Phe Pro Gln Lys Leu Lys Phe Arg 2125 2130 2135	6558
GAG CCA CAA AGG GAA GAG CGC ATA TGT CTT GTG ACA ACC AAC TTC CAA Glu Pro Gln Arg Glu Glu Arg Ile Cys Leu Val Thr Thr Asn Phe Gln 2140 2145 2150	6606
ACT AAG AGC ATG TCT AGC ATG GTG TCA GAC ACT AGT TGC ACA TTC CCT Thr Lys Ser Met Ser Ser Met Val Ser Asp Thr Ser Cys Thr Phe Pro 2155 2160 2165 2170	6654
TCA TCT GAT GGC ATA TTC TGG AAG CAT TGG ATT CAA ACC AAG GAT GGG Ser Ser Asp Gly Ile Phe Trp Lys His Trp Ile Gln Thr Lys Asp Gly 2175 2180 2185	6702
CAG TGT GGC AGT CCA TTA GTA TCA ACT AGA GAT GGG TTC ATT GTT GGT Gln Cys Gly Ser Pro Leu Val Ser Thr Arg Asp Gly Phe Ile Val Gly 2190 2195 2200	6750
ATA CAC TCA GCA TCG AAT TTC ACC AAC ACA AAC AAT TAT TTC ACA AGC Ile His Ser Ala Ser Asn Phe Thr Asn Thr Asn Asn Tyr Phe Thr Ser 2205 2210 2215	6798
GTG CCG AAA AAC TTC ATG GAA TTG TTG ACA AAT CAG GAG GCG CAG CAG Val Pro Lys Asn Phe Met Glu Leu Leu Thr Asn Gln Glu Ala Gln Gln 2220 2225 2230	6846
TGG GTT AGT GGT TGG CGA TTA AAT GCT GAC TCA GTA TTG TGG GGG GGC Trp Val Ser Gly Trp Arg Leu Asn Ala Asp Ser Val Leu Trp Gly Gly 2235 2240 2245 2250	6894
CAT AAA GTT TTC ATG AGC AAA CCT GAA GAG CCT TTT CAG CCA GTT AAG His Lys Val Phe Met Ser Lys Pro Glu Glu Pro Phe Gln Pro Val Lys 2255 2260 2265	6942
GAA GCG ACT CAA CTC ATG AAT GAA TTG GTG TAC TCG CAA GGG GAG AAG Glu Ala Thr Gln Leu Met Asn Glu Leu Val Tyr Ser Gln Gly Glu Lys 2270 2275 2280	6990

FIG. 1

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AGG AAA TGG GTC GTG GAA GCA CTG TCA GGG AAC TTG AGG CCA GTG GCT Arg Lys Trp Val Val Glu Ala Leu Ser Gly Asn Leu Arg Pro Val Ala 2285 2290 2295	7038
GAG TGT CCC AGT CAG TTA GTC ACA AAG CAT GTG GTT AAA GGA AAG TGT Glu Cys Pro Ser Gln Leu Val Thr Lys His Val Val Lys Gly Lys Cys 2300 2305 2310	7086
CCC CTC TTT GAG CTC TAC TTG CAG TTG AAT CCA GAA AAG GAA GCA TAT Pro Leu Phe Glu Leu Tyr Leu Gln Leu Asn Pro Glu Lys Glu Ala Tyr 2315 2320 2325 2330	7134
TTT AAA CCG ATG ATG GGA GCA TAT AAG CCA AGT CGA CTT AAT AGA GAG Phe Lys Pro Met Met Gly Ala Tyr Lys Pro Ser Arg Leu Asn Arg Glu 2335 2340 2345	7182
GCG TTC CTC AAG GAC ATT CTA AAA TAT GCT AGT GAA ATT GAG ATT GGG Ala Phe Leu Lys Asp Ile Leu Lys Tyr Ala Ser Glu Ile Glu Ile Gly 2350 2355 2360	7230
AAT GTG GAT TGT GAC TTG CTG GAG CTT GCA ATA AGC ATG CTC GTC ACA Asn Val Asp Cys Asp Leu Leu Glu Leu Ala Ile Ser Met Leu Val Thr 2365 2370 2375	7278
AAG CTC AAG GCG TTA GGA TTC CCA ACT GTG AAC TAC ATC ACT GAC CCA Lys Leu Lys Ala Leu Gly Phe Pro Thr Val Asn Tyr Ile Thr Asp Pro 2380 2385 2390	7326
GAG GAA ATT TTT AGT GCA TTG AAT ATG AAA GCA GCT ATG GGA GCA CTA Glu Glu Ile Phe Ser Ala Leu Asn Met Lys Ala Ala Met Gly Ala Leu 2395 2400 2405 2410	7374
TAC AAA GGC AAG AAG AAA GAA GCT CTC AGC GAG CTC ACA CTA GAT GAG Tyr Lys Gly Lys Lys Lys Glu Ala Leu Ser Glu Leu Thr Leu Asp Glu 2415 2420 2425	7422
CAG GAG GCA ATG CTC AAA GCA AGT TGC CTG CGA CTG TAT ACG GGA AAG Gln Glu Ala Met Leu Lys Ala Ser Cys Leu Arg Leu Tyr Thr Gly Lys 2430 2435 2440	7470
TTG GGA ATT TGG AAT GGC TCA TTG AAA GCA GAG TTG CGT CCA ATT GAG Leu Gly Ile Trp Asn Gly Ser Leu Lys Ala Glu Leu Arg Pro Ile Glu 2445 2450 2455	7518
AAG GTT GAA AAC AAC AAA ACG CGA ACT TTC ACA GCA GCA CCA ATA GAC Lys Val Glu Asn Asn Lys Thr Arg Thr Phe Thr Ala Ala Pro Ile Asp 2460 2465 2470	7566
ACT CTT CTT GCT GGT AAA GTT TGC GTG GAT GAT TTC AAC AAT CAA TTT Thr Leu Leu Ala Gly Lys Val Cys Val Asp Asp Phe Asn Asn Gln Phe 2475 2480 2485 2490	7614
TAT GAT CTC AAC ATA AAG GCA CCA TGG ACA GTT GGT ATG ACT AAG TTT Tyr Asp Leu Asn Ile Lys Ala Pro Trp Thr Val Gly Met Thr Lys Phe 2495 2500 2505	7662
TAT CAG GGG TGG AAT GAA TTG ATG GAG GCT TTA CCA AGT GGG TGG GTG Tyr Gln Gly Trp Asn Glu Leu Met Glu Ala Leu Pro Ser Gly Trp Val 2510 2515 2520	7710
TAT TGT GAC GCT GAT GGT TCG CAA TTC GAC AGT TCC TTG ACT CCA TTC Tyr Cys Asp Ala Asp Gly Ser Gln Phe Asp Ser Ser Leu Thr Pro Phe 2525 2530 2535	7758

FIG. 1

SUBSTITUTE SHEET

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CTC	ATT	AAT	GCT	GTA	TTG	AAA	GTG	CGA	CTT	GCC	TTC	ATG	GAG	GAA	TGG	7806
Leu	Ile	Asn	Ala	Val	Leu	Lys	Val	Arg	Leu	Ala	Phe	Met	Glu	Glu	Trp	
	2540					2545					2550					
GAT	ATT	GGT	GAG	CAA	ATG	CTG	CGA	AAT	TTG	TAC	ACT	GAG	ATA	GTG	TAT	7854
Asp	Ile	Gly	Glu	Gln	Met	Leu	Arg	Asn	Leu	Tyr	Thr	Glu	Ile	Val	Tyr	
2555					2560					2565					2570	
ACA	CCA	ATC	CTC	ACA	CCG	GAT	GGT	ACT	ATC	ATT	AAG	AAG	CAT	AAA	GGC	7902
Thr	Pro	Ile	Leu	Thr	Pro	Asp	Gly	Thr	Ile	Ile	Lys	Lys	His	Lys	Gly	
				2575					2580					2585		
AAC	AAT	AGC	GGG	CAA	CCT	TCA	ACA	GTG	GTG	GAC	AAC	ACA	CTC	ATG	GTC	7950
Asn	Asn	Ser	Gly	Gln	Pro	Ser	Thr	Val	Val	Asp	Asn	Thr	Leu	Met	Val	
		2590						2595					2600			
ATT	ATT	GCA	ATG	TTA	TAC	ACA	TGT	GAG	AAG	TGT	GGA	ATC	AAC	AAG	GAA	7998
Ile	Ile	Ala	Met	Leu	Tyr	Thr	Cys	Glu	Lys	Cys	Gly	Ile	Asn	Lys	Glu	
	2605						2610					2615				
GAG	ATT	GTG	TAT	TAC	GTC	AAT	GGC	GAT	GAC	CTA	TTG	ATT	GCC	ATT	CAC	8046
Glu	Ile	Val	Tyr	Tyr	Val	Asn	Gly	Asp	Asp	Leu	Leu	Ile	Ala	Ile	His	
	2620					2625					2630					
CCA	GAT	AAA	GCT	GAG	AGG	TTG	AGT	AGA	TTC	AAA	GAA	TCT	TTC	GGA	GAG	8094
Pro	Asp	Lys	Ala	Glu	Arg	Leu	Ser	Arg	Phe	Lys	Glu	Ser	Phe	Gly	Glu	
2635					2640					2645					2650	
TTG	GGC	CTG	AAA	TAT	GAA	TTT	GAC	TGT	ACC	ACC	AGG	GAC	AAG	ACA	CAG	8142
Leu	Gly	Leu	Lys	Tyr	Glu	Phe	Asp	Cys	Thr	Thr	Arg	Asp	Lys	Thr	Gln	
			2655						2660					2665		
TTG	TGG	TTC	ATG	TCA	CAC	AGG	GCT	TTG	GAG	AGG	GAT	GGC	ATG	TAT	ATA	8190
Leu	Trp	Phe	Met	Ser	His	Arg	Ala	Leu	Glu	Arg	Asp	Gly	Met	Tyr	Ile	
			2670					2675					2680			
CCA	AAG	CTA	GAA	GAA	GAA	AGG	ATT	GTT	TCT	ATT	TTG	GAA	TGG	GAC	AGA	8238
Pro	Lys	Leu	Glu	Glu	Glu	Arg	Ile	Val	Ser	Ile	Leu	Glu	Trp	Asp	Arg	
	2685						2690					2695				
TCC	AAA	GAG	CCG	TCA	CAT	AGG	CTT	GAA	GCC	ATC	TGT	GCA	TCA	ATG	ATT	8286
Ser	Lys	Glu	Pro	Ser	His	Arg	Leu	Glu	Ala	Ile	Cys	Ala	Ser	Met	Ile	
	2700					2705					2710					
GAA	GCA	TGG	GGT	TAT	GAC	AAG	CTG	GTT	GAA	GAA	ATC	CGC	AAT	TTC	TAT	8334
Glu	Ala	Trp	Gly	Tyr	Asp	Lys	Leu	Val	Glu	Glu	Ile	Arg	Asn	Phe	Tyr	
2715					2720				2725						2730	
GCA	TGG	GTT	TTG	GAA	CAA	GCG	CCG	TAT	TCA	CAG	CTT	GCA	GAA	GAA	GGA	8382
Ala	Trp	Val	Leu	Glu	Gln	Ala	Pro	Tyr	Ser	Gln	Leu	Ala	Glu	Glu	Gly	
				2735					2740					2745		
AAG	GCG	CCA	TAT	CTG	GCT	GAG	ACT	GCG	CTT	AAG	TTT	TTG	TAC	ACA	TCT	8430
Lys	Ala	Pro	Tyr	Leu	Ala	Glu	Thr	Ala	Leu	Lys	Phe	Leu	Tyr	Thr	Ser	
			2750					2755					2760			
CAG	CAC	GGA	ACA	AAC	TCT	GAG	ATA	GAA	GAG	TAT	TTA	AAA	GTG	TTG	TAT	8478
Gln	His	Gly	Thr	Asn	Ser	Glu	Ile	Glu	Glu	Tyr	Leu	Lys	Val	Leu	Tyr	
	2765						2770					2775				
GAT	TAC	GAT	ATT	CCA	ACG	ACT	GAG	AAT	CTT	TAT	TTT	CAG	AGT	GGC	ACT	8526
Asp	Tyr	Asp	Ile	Pro	Thr	Thr	Glu	Asn	Leu	Tyr	Phe	Gln	Ser	Gly	Thr	
	2780						2785					2790				

FIG. 1

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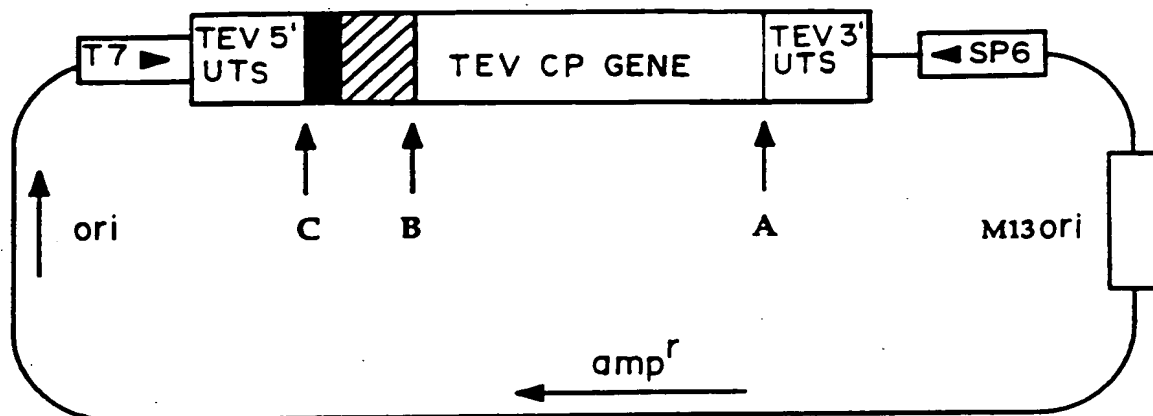
GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT	8574
Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp	
2795 2800 2805 2810	
AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT	8622
Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr	
2815 2820 2825	
TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT	8670
Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu	
2830 2835 2840	
CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT	8718
Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu	
2845 2850 2855	
TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA	8766
Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr	
2860 2865 2870	
CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA	8814
His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly	
2875 2880 2885 2890	
GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG	8862
Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp	
2895 2900 2905	
TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG	8910
Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met	
2910 2915 2920	
ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA	8958
Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu	
2925 2930 2935	
AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG	9006
Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu	
2940 2945 2950	
GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT	9054
Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro	
2955 2960 2965 2970	
AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT	9102
Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr	
2975 2980 2985	
GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG	9150
Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg	
2990 2995 3000	
GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT	9198
Glu Ala His Met Gln Met Lys Ala Ala Val Arg Asn Ser Gly Thr	
3005 3010 3015	
AGG TTA TTT GGT CTT GAT GGC AAC GTG GGT ACT GCA GAG GAA GAC ACT	9246
Arg Leu Phe Gly Leu Asp Gly Asn Val Gly Thr Ala Glu Glu Asp Thr	
3020 3025 3030	
GAA CGG CAC ACA GCG CAC GAT GTG AAC CGT AAC ATG CAC ACA CTA TTA	9294
Glu Arg His Thr Ala His Asp Val Asn Arg Asn Met His Thr Leu Leu	
3035 3040 3045 3050	

FIG. 1

GGG GTC CGC CAG TGA TAGTTTCTGC GTGTCTTTGC TTTCCGCTTT TAAGCTTATT	9349
Gly Val Arg Gln	
GTAATATATA TGAATAGCTA TTCACAGTGG GACTTGGTCT TGTGTTGAAT AGTATCTTAT	9409
ATATTTTAAT ATGTCTTATT AGTCTCATTA CTTAGGCGAA CGACAAAGTG AGGTCACCTC	9469
GGTCTAATTC TCCTATGTAG TCGGAG	9495

FIG. 1

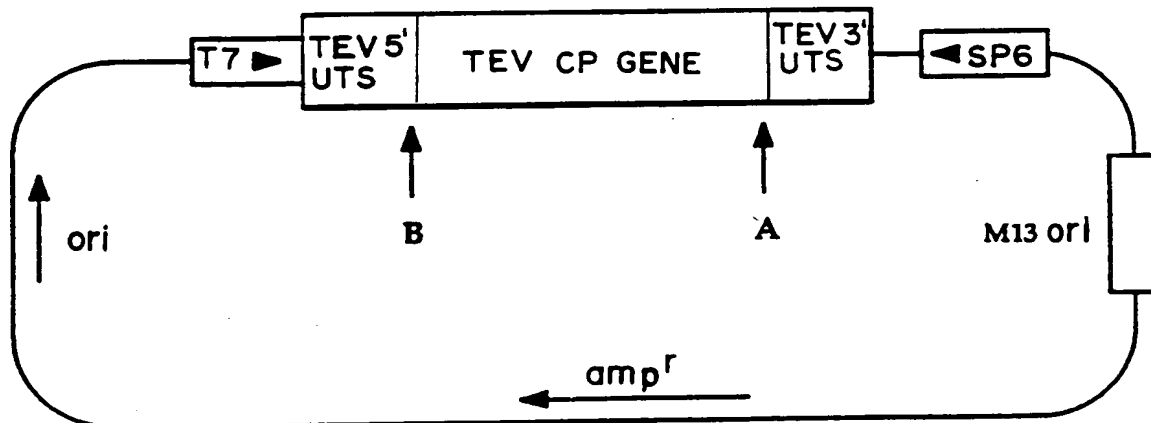
14/16



pTL 37/8595

1. GENERATE BamHI SITE AT A (nt 9312-9317)
2. GENERATE NcoI SITE AT B (nt 8516-8521)
3. GENERATE BamHI SITE (nt 133-138) NcoI SITE (nt 143-148) AND DEOXYADENYLATE RESIDUE (at nt 142) at C.

DIGEST WITH NcoI
 REMOVE TEV NUCLEOTIDES 143-200/8462-8516
 (FLANKED BY SITES B AND C) AND RELIGATE.



pTC:FL

FIG. 2

**Product in
Transgenic Plants**

Plant Line

**TEV Coat Protein Gene Constructs Inserted
into *Nicotiana tabacum* cv. Burley 49**

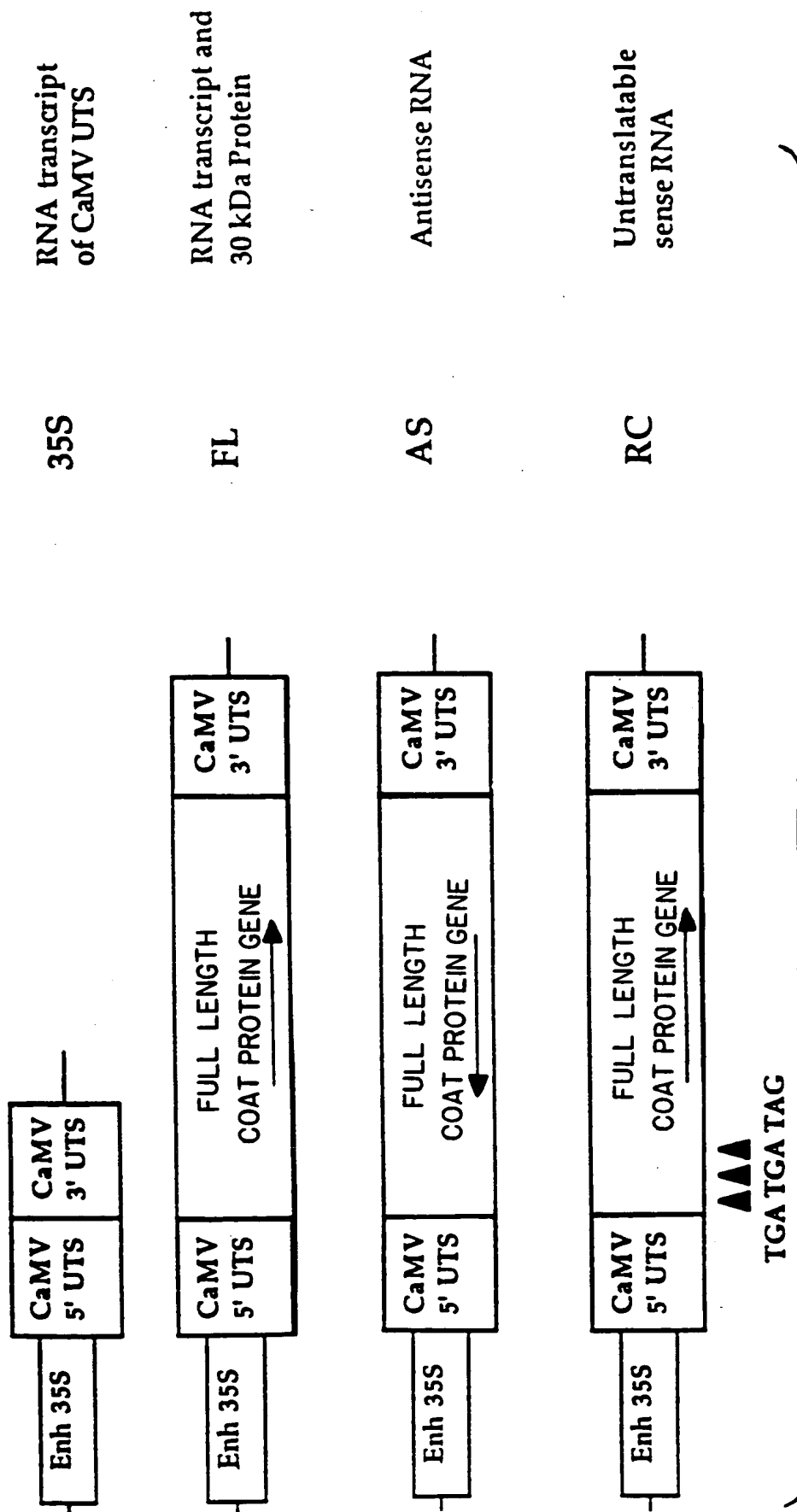
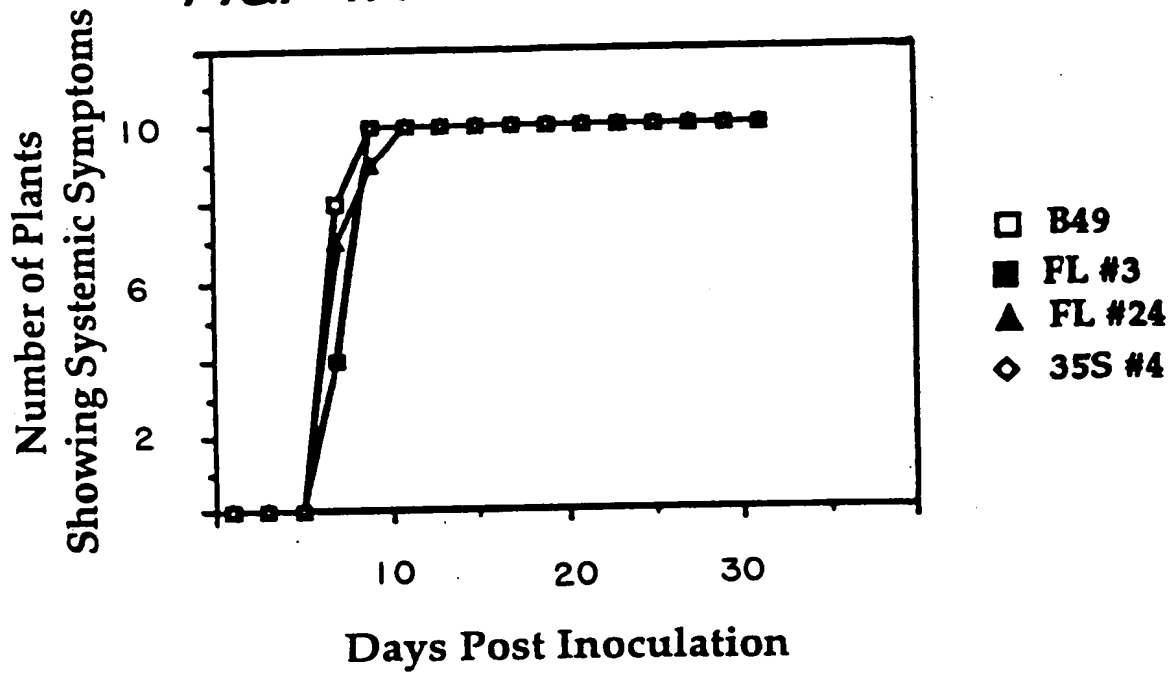
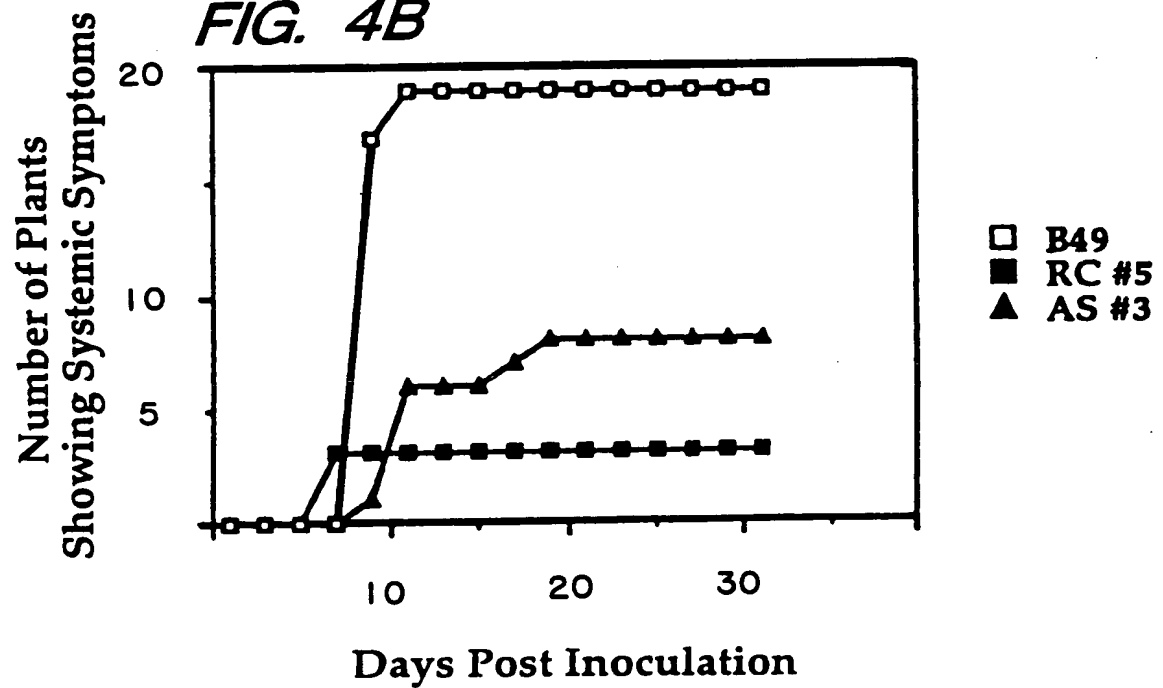


FIG. 3

FIG. 4A**FIG. 4B**

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/01544**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : C12N 1/21, 5/10, 15/33, 15/82; C07H 21/04; A01H 5/00

US CL : 435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG,

search terms: virus or viral, untranslat?, resistan?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Molecular Plant-Microbe Interactions, Volume 5, No. 2, issued March 1992, Lindbo et al, "Pathogen-derived resistance to a potyvirus: immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence", pages 144-153, see entire document.	1-27
X,P	Virology, Volume 189, No. 2, issued August 1992, Lindbo et al, "Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts", pages 725-733, see entire document.	1-27

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 May 1993

Date of mailing of the international search report

26 MAY 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Authorized officer

P. MOODY

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/01544**A. CLASSIFICATION OF SUBJECT MATTER**

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A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
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